Microbial impact on phytoremediation at heavy metal contaminated soils

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1 Introduction

Soil is a very specific component of terrestrial ecosystems, constituting a habitat for microbes, plants and human beings. This dynamic biological system is essential for the growth of plants and their existence by providing nutrients and thus strongly influences the overall life on earth. But it also acts as geochemical sink for hazardous substances. The input of toxic inorganic contaminants in surface soils, such as heavy metals and metalloids, are likely to increase with increasing industrial and mining activities (Hohl and Varma 2010). In contrast to organic pollutants, heavy metals are not degradable and can persist for several decades in the local environment and thus, posing potential toxicity problems. Once accumulated in soils, they can also be distributed to other environmental compartments, which negatively affect their containment and remediation (Christensen et al. 1994, Tchounwou et al. 2012). The fate of metal contaminants in soils depends on various processes, including dissolution, sorption, complexation and precipitation or adsorption by soil microorganisms and plant roots. In addition, all these processes strongly affect their behaviour and bioavailability in soil system (Alloway 1995, Rieuwerts et al. 1998). Hence, the remediation of metal-contaminated soils is of high importance. Nowadays, a high number of remediation methods exist based on physical, chemical, thermal/electrical or biological techniques. Most conventional soil cleaning methods are not satisfying and are both disruptive and expensive (Arthur et al. 2005, Mulligan et al. 2001). Alternatively, phytoremediation provides a sustainable and less costly solution for remediating heavy metal-contaminated soils.

1.1 Heavy metal contamination in soils

The release of heavy metals to the soil environment has accelerated dramatically since the beginning of industrial revolution in the 19th century. Soil contamination with heavy metals and their associated toxic effects on environmental and human health is becoming a serious problem not only in industrialized countries (Jadia and Fulekar 2009). Unlike organic pollutants, heavy metals cannot be degraded and persist for a long time after their introduction (Adriano et al. 2004). Heavy metal contamination of soil can originate from both natural (geogenic) and anthropogenic processes. The main natural source of heavy metals and metalloids is geologic parent material including igneous and sedimentary rocks. Weathering, the basic soil-forming process, and pedogenic processes play a significant role in the metal composition and concentration of parent rocks and metallic

minerals. Biochemical weathering leads to the destruction of parent material and to the release of metals contained in soil minerals into soil solution.

High loads of trace elements such as Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb generally occur in continental crust and surface soils from parent material (Nagajyoti et al. 2010). For instance, ultramafic rocks, such as serpentinite, contain more than 70 % ferromagnesian or mafic materials. Serpentine soils are formed by the weathering of ultramafic rocks and are characterized by a low Ca:Mg ratio, low content of plant-available macronutrients (P,K,N) and toxic levels of heavy metals such as Ni, Cr and Co (Brady et al. 2005, Kazakou et al. 2008). Additionally, the lack of organic material and the low moisture-holding capacity are limiting factors for plant growth on serpentine (ultramafic) soils. Similarly, seleniferous rocks like black shales, carbonaceous limestones and Se-rich coal are a major source of Se enrichment in soil sediments (Frankenberger and Arshad 2001).

Other important natural sources for heavy metal input to soil environment include volcanic activities, marine aerosols and airborne emissions from forest and prairie fires. Volcanoes have been reported to emit high contents of natural Al, Zn, Pb, Cu and Hg to the atmosphere (Pyle and Mather 2003, Varekamp and Buseck 1986). Marine aerosols also contribute to the transport of toxic metals in many environments, particularly the transport of dusts originating from the Sahara (Erel and Torrent 2010). Natural wildfires, such as bushfires in drought affected areas, exert an important influence in the emission of volatile heavy metals like Hg and Se into soil and atmosphere.

Beside their natural availability in soil environments, anthropogenic activities are an increasing problem for heavy metal accumulation in urban soils. Anthropogenic sources of soil metal contamination are primarily associated with metalliferrous mining and smelting, industrial processes, agricultural chemicals, disposal of municipal waste, domestic effluents and atmospheric deposition (Ross 1994). Industrial sources of metal contamination mainly include mining operations, metal and plastic processing, coal combustion, refinement and manufacturing. One of the major causes for large quantities of heavy metals and metalloids in soil environments is the mining industry. Specifically, mining of non-ferrous ore minerals, including gold, copper and uranium, is associated with acid mine drainage (AMD) that is produced when sulfide minerals, particularly pyrite or pyrrhotite, are exposed to both oxygen and water (Fig. 1; Johnson and Hallberg 2005). Conditions required for AMD generation are: (1) oxygen from the atmosphere or another oxidant from chemical sources, (2) an aqueous environment and (3) the presence of iron or sulfide oxidizing bacteria. The activity of oxidizing bacteria such as *Acidithiobacillus ferrooxidans* or *Gallionella ferruginea* plays an important role in accelerating this process up to 1,000,000 times (Akcil and Koldas 2006, Banks et al. 1997, Haferburg et al. 2007b).

As a consequence of iron pyrite oxidation during mining activities produced sulphuric acid solubilizes toxic heavy metals and, thus may lead to metal input into surface and groundwater through seepage waters as well as contamination of soils at active and abandoned mine sites (Peppas et al. 2000). Although formation of AMD occurs naturally, the increasing release of such acidic metal-rich mine effluents constitutes a serious environmental problem in many countries worldwide. Hence, the prevention of the acid-generating process and/or controlled placement of acid-generating waste should be of high importance (Hilson and Murck 2001, Kuyucak 2002).

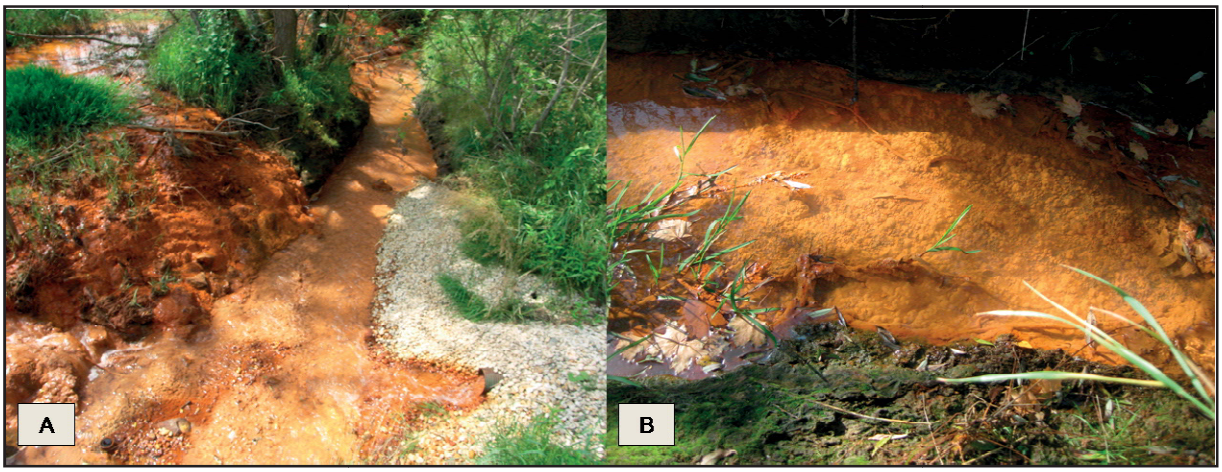

Figure 1: (A) AMD formation and inflow into creek Gessenbach at the former uranium mining site, WISMUT, in Eastern Thuringia, Germany. (B) Precipitated iron hydroxides in creek sediment.

Agricultural sources of heavy metal input to harmful concentrations in arable soils comprise the excessive use of organic and inorganic fertilizers, sewage sludge or biosolids, metal-based pesticides and manures from intensive animal production (Nagajyoti et al. 2010). Inorganic fertilizers are ubiquitous sources of increased soil metal loads by the fact that they do not only contain plant growth promoting macronutrients like N, P, K, Ca and Mg but also significant concentrations of heavy metals. For instance, phosphate fertilizers contain a wide range of toxic heavy metals including Cd, U, As, V and Zn and their repeated use can have a considerable acidifying effect on soils resulting in the mobilization and accumulation of heavy metal contaminants (Alloway and Ayres 1998, Nicholson et al. 2003, Nriagu 1988). In addition, micronutrient fertilizers supply relatively high levels of Cu and Zn to soils in regions of intensive farming (Nicholson et al. 2010).

Sewage sludges are the insoluble residues from the treatment of domestic and industrial waste waters which are frequently recycled to arable soil to improve crop production. However, their beneficial characteristics are limited by their large amounts of heavy metals in varying concentrations. The most common heavy metals found in sludge amended soils are Cd, Cu, Ni and Zn originated primarily from domestic sources including human excreta, health care products, domestic "grey" water and industrial waste waters (Haynes et al. 2009, Sánchez-Martín et al. 2007). The application of metal-containing fungicides and insecticides, which are used in large quantities on field and plantation crops, constitutes another important non-point source of metal enrichment in agricultural soils. Especially copper-based fungicides were found to contribute to significant accumulation of Cu in soils of vineyards and orchards in many countries (Hildebrandt et al. 2008, Zhou et al. 2011). The addition of livestock manures, particularly pig and poultry, has been recognized as most important input to soils in European countries for Cu and Zn, respectively (Nicholson et al. 1999, Park et al. 2011). In contrast to localized soil contamination heavy metals from atmospheric deposition often affect large areas. The emitted heavy metals are usually present as aerosol particles with an average residence time of 10 to 30 days and transported hundreds of kilometres away from the source of emission (Bowen 1979, Nagajyoti et al. 2010). Inputs of heavy metals from atmospheric deposition include coal and oil-fired electricity generation stations, combustion of municipal wastes, smelters and foundries, automobile exhausts, smoke and other emissions from industrial and domestic heating (Gray et al. 2003, Nagajyoti et al. 2010).

1.2 Biological effects of heavy metals

The term "heavy metal" has been used in various publications since many years, but there is still no authoritative definition to be found in the relevant literature. Most definitions of heavy metals are based upon the density (specific gravity), atomic weight or mass, atomic number or on other chemical properties. The term "heavy metal" is often associated to the group of metals and metalloids of relatively high atomic density (> 5 g/cm^3) and an atomic number above 20 (Gardea-Torresdey et al. 2005, Hodson 2004, Nies 1999, Phipps 1981). From a biological point of view, heavy metals should be defined in relation to their chemical properties rather than on atomic density. Hence, their classification based on the position in the periodic table include (1) transition elements, all elements of groups 3-12; (2) rare earth elements, which are subdivided into lanthanides and actinides and (3) some elements from the p-block that are either metals or metalloids (Al, Pb, Sb) (Duffus 2002). Some of these metals are crucial as essential elements (micronutrients) for various biochemical and physiological processes at low concentrations, whereas metals such as

Al, Cd, Pb, Hg and U have no biological role and are considered as non-essential metals (Bruins et al. 2000, Tchounwou et al. 2012). Essential metals including Co, Cu, Fe, Mn, Ni, Zn, Mg, K, and Ca serve as catalysts in biochemical reactions or cofactors of many enzymes and are involved in numerous oxidation-reduction processes. Zinc and Cu for example are required for the catalytic activity of many oxidative stress-related enzymes (catalases, dehydrogenases, peroxidases, oxidases and superoxide dismutases) and play a key role in metabolism of living cells (Ahemad 2014, Ji and Silver 1995, Nies 1992, Stern 2010). Nevertheless excessive concentrations of both essential and non-essential metals are toxic. But the toxicity of a certain metal varies greatly from organism to organism and depends on its speciation and bioavailability as well as other abiotic factors such as pH, temperature or organic materials (Abbas and Edwards 1989, Babich and Stotzky 1983). Heavy metal toxicity may result from alterations in the conformation structure of nucleic acids and proteins, inhibition of enzymes, blocking functional groups of metabolically important molecules, displacement or substitution of essential ions and lead to enhanced production of reactive oxygen species (ROS) (Hall 2002, Rascio and Navari-Izzo 2011, Schmidt et al. 2005). Consequently, elevated concentrations of heavy metals in soils not only negatively affect soil fertility and plant growth but also decrease soil microbial biomass and activity (Giller et al. 1998, Rajapaksha et al. 2004).

1.3 Bioavailability of heavy metals

To be bioavailable, the metal contaminant has to come in direct contact with the organism (physical accessibility) and has to be in a soluble form (chemical accessibility) for uptake (Adriano et al. 2004, Bolan et al. 2014). The bioavailability of heavy metals in soils is influenced by (1) soil conditions including pH, redox potential (Eh), soil texture, content of organic matter and clay minerals, Fe- and Mn-(oxy)hydroxides, presence and concentration of organic/inorganic complex forming anions, water capacity and temperature, (2) metal speciation and solubility and (3) biological activities such as exudation of metal chelators released by plant and/or soil microorganisms (Fischerová et al. 2006, Pilon-Smits 2005, Violante et al. 2010). Moreover, metal bioavailability strongly depends on their transfer from the solid to the solution phase in soils. Processes affecting the solid-solution partition and, thus bioavailability of soil metals are cation exchange, specific adsorption, precipitation and complexation. Most metals are usually present as cations in the soil solution. Exchange of metal cations depends on the density of negative charges on the surfaces of soil particles and on the charges of metal ions in solution and those on the soil surface. In order to maintain electroneutrality, the surface negative charge is balanced by an equal quantity of cations from the soil solution. This exchange of

ions is reversible and diffusion controlled (Brown 1954, Evans 1989). The specific adsorption involves the exchange of cationic and anionic metal ions with surface ligands to form partly covalent bonds with charged surfaces of soil components such as hydrous oxides of Al, Fe and Mn. This process largely depends on soil pH and the capability of metal ions to form hydroxyl complexes (Bolan et al. 2014, Jarvis and Jones 1980).

Despite adsorption, formation of insoluble metal precipitates (mainly as phosphates, carbonates or sulphates) is one of the predominant processes in soils with high pH and large quantities of heavy metal ions which affects solubility of heavy metals (Naidu et al. 1997, Rieuwerts et al. 1998). In addition, complexation of metal cations with organic or inorganic ligands can either directly or indirectly alter the bioavailability of soil metals (Evans 1989, Ström et al. 2005). Most of the soil processes that influence the solid-solution partitioning of heavy metals are pH dependent. Metal solubility tends to increase under acidic conditions and decrease at higher pH values (Chuan et al. 1996). At low pH, cationic heavy metal sorption onto charged solid surfaces is generally weaker than at neutral pH and increases in slightly alkaline conditions. At alkaline pH, surface adsorption is reduced due to organic and inorganic complexation of metal cations in soil solution (Schuwirth and Hofmann 2006). Additionally, soil pH is closely linked to changes in redox potential (Eh). Redox reactions in soils also significantly affect metal solubility due to changing their redox state and by the dissolution of hydrous Al, Mn and Fe oxides, resulting in the release of co-precipitated metals into the soil solution (Chuan et al. 1996, Hindersmann and Mansfeldt 2014). In contrast, under reducing conditions sulphate ions are reduced to sulphide, which can lead to the formation of insoluble metal sulfides, such as CdS, CuS, MnS and ZnS (Blume et al. 2010). The soil texture including secondary minerals such as phyllosilicates, clay minerals, Fe- and Mn(oxy)hydroxides and organic matter content constitute other important abiotic factors controlling bioavailability of heavy metals in soil (Rieuwerts et al. 1998).

The bioavailability of heavy metals can also be altered by soil microorganisms, mycorrhizal fungi and plants through reducing soil pH, release of iron chelators and siderophores, changes in redox conditions or solubilization of metal phosphates (Abou-Shanab et al. 2008, Zhuang et al. 2007). The excretion of low molecular weight, iron-chelating substances - siderophores, which are involved in iron acquisition, has mostly been described for bacteria and fungi, but also for some plants (Crowley et al. 1991). These chelating molecules form high affinity complexes with Fe^{3+}, but also interact with other metal cations (e.g., Fe^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}), albeit with reduced affinity (Boukhalfa and Crumbliss 2002, Fernandez and Winkelmann 2005, Rajkumar et al. 2010). Siderophores are excreted by both bacteria (e.g., *Streptomyces acidiscabies*) and

mycorrhizal fungi (e.g., *Suillus luteus*) and in most cases, catecholate and hydroxymate siderophores are used (Ahmed and Holmström 2014, Dimkpa et al. 2008, Haselwandter et al. 2011).

While most plants ensure iron supply through acidification of the rhizosphere via H^+ extrusion from the roots or by excretion of organic acids such as oxalate, citrate or malate, graminaceous plants like *Sorghum* are able to produce phytosiderophores in their roots (Jadia and Fulekar 2008, Lasat 2002, Zhuang et al. 2007). Exudation of organic acids by either rhizosphere microbes or plant roots may affect metal behaviour in soils by forming metal complexes or decreasing the pH and thus, increase their mobility (Park et al. 2011, Ryan et al. 2001). Additionally, microbially produced surfactants (biosurfactants) can also impact significantly on metal solubility and bioavailability in soil solution (Braud et al. 2006). Cell wall adsorption or binding of metals to extracellular polymeric substances (EPS) including polysaccharides, proteins and humic substances further strongly influence the available metal concentration. In fact, sorption by microbial or root cell walls allows metal sequestration and thus, contributes to their immobilization in soils (Haferburg and Kothe 2007, Haferburg et al. 2007a, Hall 2002). Certain plant growth promoting bacteria (PGPB) are able to alter the rhizosphere soil pH through oxidation or reduction reactions, resulting in an either increased or decreased metal mobility (Di Gregorio et al. 2005, Shi et al. 2011). The soil is a dynamic system and changes in its properties remarkably affect the speciation and bioavailability of heavy metals, which need to be considered in the remediation of metal contaminated sites.

1.4 Phytoremediation

Conventional methods of metal remediation, such as excavation and landfilling, or physical and chemical soil treatments including soil washing or extraction are expensive, disturb soil properties, can cause secondary pollutants and are limited to relatively small areas (Arthur et al. 2005, Khan 2005). In contrast, an environmentally friendly and cost-efficient strategy is phytoremediation – the use of metal-accumulating plants to clean-up and restore low to moderate contaminated areas (Pilon-Smits 2005). Phytoremediation represents a green solution to treat soil contamination having several advantages over common remediation techniques. Benefits of this solar-driven and passive remediation technology are that it can be performed in situ, prevents erosion and metal leaching by a vegetative ground cover, allows for site restoration and offers the possibility of bio-recovery of the heavy metals. But the main advantages are the low costs and its application at very large field sites (LeDuc and Terry 2005, Lee 2013, McGrath et al. 2001).

Different phytoremediation approaches can be considered, depending on the pollutant and its concentration as well as present site conditions (see Fig. 2).

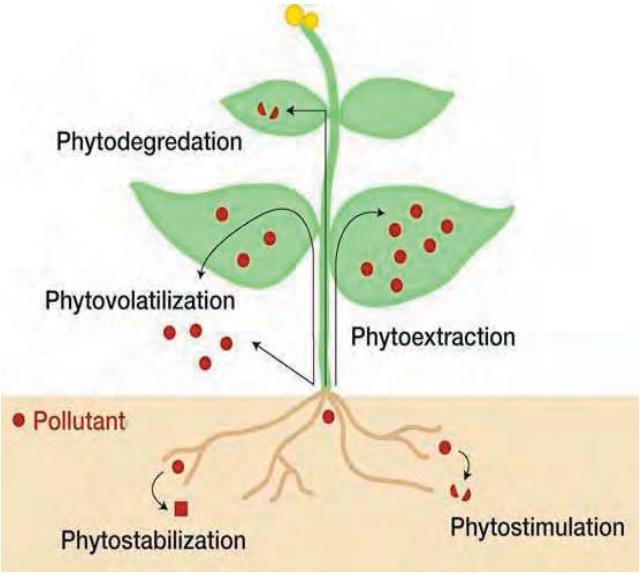

Figure 2: Different mechanisms of phytoremediation (Pilon-Smits 2005)

The main processes applied in the removal of heavy metals from contaminated soil are phytoextraction (also known as phytoaccumulation) and phytostabilization. Phytoextraction aims at removing heavy metals from soil through uptake by plant roots as well as their translocation and accumulation into aboveground biomass, especially into plant shoots, followed by harvest and treatment of contaminated plant biomass (Garbisu and Alkorta 2001, Raskin et al. 1997). The success of phytoextraction is mainly influenced by the toxicity level and bioavailability of contaminants and the plant species. Important plant characteristics for an efficient metal extraction are high tolerance to metals, fast growth rate and high biomass production, deep, well-branched root system, high root-to-shoot-transport and accumulation into shoot biomass, adaptability to environmental conditions and easily harvestable (Ernst 2005). Depending on the selected plant species, there are two main strategies for metal phytoextraction. One possibility is the use of metal hyperaccumulator species like *Thlaspi caerulescens* (Zn/Cd hyperaccumulator), which can accumulate specific metals at concentrations of more than 0.1 % of their dry weight without symptoms of phytotoxicity but their slow growth and low biomass production tends to limit their phytoextraction ability (Escarré et al. 2000, Sheoran et al. 2011). The other possible strategy includes the use of non-accumulator plants either high biomass producing crop species or fast-growing, highly productive trees. In recent years, the

potential of metal-tolerant field crops (mainly *Asteraceae*, *Brassicaceae* or *Poaceae*) or woody plant species (especially *Salix* and *Populus* species) for metal extraction purposes has been extensively investigated (Brunetti et al. 2011, Ebbs and Kochian 1998, Marchiol et al. 2007, Murakami and Ae 2009, Robinson et al. 2000, Unterbrunner et al. 2007). Their high-yielding ability can compensate for a lower metal accumulation capacity, resulting in similar or even higher metal concentration in their tissues than hyperaccumulators. In addition, harvested biomass containing accumulated heavy metals are environmental acceptable sources for bioenergy production (Licht and Isebrands 2005, Masarovicova et al. 2009). Unlike phytoextraction, phytostabilization aims on immobilization of metal contaminants by surface adsorption/accumulation in roots to reduce their mobility and bioavailability, thus limiting their diffusion in soil (Mendez and Maier 2008).

The established vegetation cover contributes to a long-term stabilization of metals but also prevents soil erosion and metal leaching into groundwater. The stabilization of metals can be enhanced by root-released chelating substances as well as high contents of clay minerals or organic matter in soils (Vangronsveld et al. 2009). Favorable plant properties include high capacity to retain contaminants in roots or rhizosphere, low shoot-to-root metal concentration ratio and high transpiration rates. In contrast to phytoextraction, plants used for phytostabilization should be metal excluders, which restrict entry and transport of metals into their aerial parts (Brunner et al. 2008, Kucharski et al. 2005). The revegetation of mine tailings and metal contaminated sites with a combination of fast-transpiring trees and perennial grasses constitutes a cost-effective and environmental sustainable remediation method. However, stabilization (immobilization) of metal contaminants by plants is not a permanent solution because the heavy metals remain in the soil environment.

Microbially supported phytoremediation, using heavy metal-resistant soil bacteria and mycorrhizal fungi, has been shown to positively affect plant performance and metal uptake on heavy metal-contaminated soils (Chen et al. 2013, Nicoara et al. 2014, Nogueira et al. 2007). The activity of soil microorganisms significantly influences metal bioavailability in the rhizosphere, resulting in an either improved metal uptake into plant biomass due to metal solubilization and transfer, or in an immobilization of metals in soil due to intracellular or extracellular complexation (Glick 2010, Haferburg et al. 2007a, Kothe et al. 2010, Kuffner et al. 2008, Langella et al. 2014, Schindler et al. 2012). They stimulate plant growth through mobilization of nutrients in soil, such as nitrogen, phosphorus and iron, secretion of phytohormones and siderophores or indirectly by decreasing the inhibitory effects of plant pathogens (Ahemad and Kibret 2014, Weyens et al. 2009). Heavy metal-resistant soil bacteria, specifically Gram-positive streptomycetes, could be shown to

enhance plant growth under heavy metal stress by production of growth-promoting hormones like indole acetic acid (IAA) or siderophore production. For instance, hydroxamate siderophores produced by nickel-resistant *Streptomyces acidiscabies* E13 has been found to protect cowpea plants from oxidative stress by lowering the formation of free radicals and supply plants with sufficient iron, resulting in an improved growth in highly contaminated soil. In addition, these elicited iron chelators are able to protect microbial auxins from degradation (Dimkpa et al. 2008, Dimkpa et al. 2009). Moreover, there is evidence that mycorrhization of plant roots by arbuscular mycorrhizal fungi, mostly of the genus *Glomus*, increases nutrient acquisition and contributes to plant growth as well as protection against heavy metal toxicity in metal polluted habitats (Khan et al. 2000, Leyval et al. 1997, Liang et al. 2009). These plant growth promoting attributes of soil microorganisms and their interactions with plant roots within the rhizosphere may facilitate phytoremediation of metalliferrous soils.

1.5 Research objectives

The main objective of this work was to evaluate the role of extremely heavy metal-resistant *Streptomyces* strains, isolated from the former uranium mining area near Ronneburg (Eastern Thuringia) and the arbuscular mycorrhizal fungus *Rhizophagus irregularis* in microbially assisted phytoremediation of heavy metal-contaminated soils. The impact of microbial application on remediation potential of metal-tolerant, high biomass crop species was investigated in pot experiments and at field scale using metal-contaminated soil substrate from the test site Gessenwiese. Therefore, this work evaluated the effects of microbial inoculants on (1) plant growth promotion in multi-metal contaminated substrate; (2) metal extraction into harvestable biomass as well as metal stabilization in soil by the grasses *Andropogon gerardii* and *Sorghum bicolor*, and the herbaceous plant *Silphium perfoliatum* and (3) alteration of heavy metal bioavailability in soil at different experimental scales.

2 Summary of manuscripts

2.1 Microbially supported phytoremediation of heavy metal contaminated soils: strategies and applications

Phieler R, Voit A, Kothe E (2014): Microbially supported phytoremediation of heavy metal contaminated soils: strategies and applications. In: Schippers A, Glombitza F, Sand W (Eds.), Geobiotechnology I. Advances in Biochemical Engineering/Biotechnology, Springer Berlin Heidelberg, 211-235

Summary

Soil contamination by heavy metals in industrial and post-mining areas constitutes a major environmental problem. Remediation of such contaminated areas by conventional techniques are both expensive and site destructive. Alternatively, phytoremediation provides a sustainable and gentle method to clean-up and restore heavy metal-contaminated sites. This article reviewed the potential of different plant-based remediation approaches including phytoextraction and phytostabilization with focus on plant-microbe-interactions in the rhizosphere. Here, the potential of phytoremediation assisted by soil microorganisms are discussed. Thus, the impact of heavy metal-resistant streptomycetes and arbuscular mycorrhiza on remediation potential and heavy metal uptake by plants are investigated in a field-scale experiment. The results showed that heavy metal-resistance streptomycetes and arbuscular mycorrhiza are a useful tool in supporting phytoremediation strategies of metal-contaminated land by altering heavy metal bioavailability in soil and their plant growth promoting effects.

Contributions of the authors

R. Phieler: Conception and preparation of the manuscript, design of figure and tables

A. Voit: Preparation of the chapter “Rhizosphere Interactions”

E. Kothe: Preparation of chapters “Case Study” and “Conclusion”, supervision and correction of the entire manuscript

Personal contribution to this manuscript: 65 %

2.2 Phytoremediation using microbially mediated metal accumulation in *Sorghum bicolor*

Phieler R, Merten D, Roth M, Büchel G, Kothe E (2015): Phytoremediation using microbially mediated metal accumulation in *Sorghum bicolor*. Environmental Science and Pollution Research, 1-9

Summary

The application of microbial amendments on heavy metal accumulation by the high-biomass crop *Sorghum bicolor* in metal-contaminated soil was evaluated. In pot experiments and with field trials the impact of two heavy metal-resistant *Streptomyces* strains and the arbuscular fungus *Rhizophagus irregularis* on the alteration of metal mobility, plant performance and metal extraction capacity of *S. bicolor* was investigated. It was shown that biomass production and metal uptake by inoculated plants was enhanced under controlled conditions, while in the field the impact of microbes was less clear. In addition, microbial inoculation significantly decreased bioavailability of heavy metals in potting substrate, whereas due to heterogenous soil conditions at field scale neither plant growth nor inoculation induced visible changes.

Contributions of the authors

R. Phieler: Conception and design of experiments, laboratory and field work, evaluation of data, preparation of manuscript

D. Merten: Analysis of heavy metal contents in soil and plant digestions by ICP-MS and ICP-OES

M. Roth: Fermentation of bacterial strains

G. Büchel: Co-supervision of the project

E. Kothe: Supervision of the project, discussion of results, correction of the manuscript

Personal contribution to this manuscript: 75 %

2.3 Impact of soil microbes on remediation potential of high biomass crop plants

Phieler R, Funai B, Fürst D, Merten D, Kothe E (submitted to Journal of Hazardous Materials)

Summary

In a greenhouse study the effects of metal-resistant *Streptomyces* strains and the arbuscular fungus *Rhizophagus irregularis* on soil metal availability, plant performance and phytoremediation efficiency of the grasses *Andropogon gerardii* and *Sorghum bicolor*, and the herbaceous high yielding crop *Silphium perfoliatum* were investigated. Microbial inoculation increased biomass productivity, but had variable effects on metal accumulation into plant shoots. Bacterial and mycorrhizal inoculation was found to decrease bioavailable fractions of most metals in the respective substrate.

Contributions of the authors

R. Phieler: Conception and design of experiments, laboratory work, evaluation of data, preparation of manuscript

B. Funai: DNA isolation and PCR

D. Fürst: Assistance during the plant harvest

D. Merten: Analysis of heavy metal contents in soil and plant digestions by ICP-MS and ICP-OES

E. Kothe: Supervision of the project, discussion of results, correction of the manuscript

Personal contribution to this manuscript: 75 %

Ort, Datum	Unterschrift des Doktoranden
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3 Manuscripts

3.1 Manuscript I

Microbially supported phytoremediation of heavy metal contaminated soils: strategies and applications

Phieler R, Voit A, Kothe E

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Microbially Supported Phytoremediation of Heavy Metal Contaminated Soils: Strategies and Applications

René Phieler, Annekatrin Voit and Erika Kothe

Abstract Heavy metal contamination of soil as a result of, for example, mining operations, evokes worldwide concern. The use of selected metal-accumulating plants to clean up heavy metal contaminated sites represents a sustainable and inexpensive method for remediation approaches and, at the same time, avoids destruction of soil function. Within this scenario, phytoremediation is the use of plants (directly or indirectly) to reduce the risks of contaminants in soil to the environment and human health. Microbially assisted bioremediation strategies, such as phytoextraction or phytostabilization, may increase the beneficial aspects and can be viewed as potentially useful methods for application in remediation of low and heterogeneously contaminated soil. The plant–microbe interactions in phytoremediation strategies include mutually beneficial symbiotic associations such as mycorrhiza, plant growth promoting bacteria (PGPB), or endophytic bacteria that are discussed with respect to their impact on phytoremediation approaches.

Keywords Microbially assisted remediation · Phytomining · Phytoremediation

Abbreviations

Cys	Cysteine
Glu	Glutamine
Gly	Glycine
MT	Metallothionein
PC	Phytochelatin
PGPB	Plant growth promoting bacteria
ROS	Reactive oxygen species

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Contents

1 Introduction

Approximately 1,400,000 sites in Western Europe are highly contaminated with heavy metals [1]. The increasing release of heavy metals and metalloids to the environment due to anthropogenic activities such as mining and smelting operations, burning of fossil fuels, municipal wastes, and agrochemical usage is a serious problem worldwide [2–4]. Soil contamination by metals can lead to loss of soil functions such as buffering, filtering, and transforming capacities, and may lead to contamination of ground and surface waters [5]. Toxic levels of heavy metals in soil are also a potential risk for environmental and human health due to soil-to-plant transfer of metals and their accumulation in animal or human bodies through the food chain [6, 7].

The term heavy metal includes elements with an atomic density greater than 6 g/cm^3 and a specific gravity above five [8]. Some of these metals play an important role as essential elements in biochemical reactions, whereas metals such as Cd, Pb, or As are not essential. The essential metals such as Cu, Fe, Mn, Ni, Zn, Mg, K, and Ca are required in low concentrations as nutrients [9]. They serve as catalysts in biochemical processes or cofactors of many enzymes and are involved in numerous physiological processes. But concentrations exceeding a threshold nevertheless are toxic, as every metal may cause alterations in the conformation structure of nucleic acids and proteins, inhibit enzymes, block functional groups of important molecules, and lead to production of reactive oxygen species (ROS; [10–12]). In contrast to organic pollutants, heavy metals cannot be degraded and their mobility in soil is influenced by soil conditions, metal speciation, and solubility in water. Metal availability in the water phase and hence their bioavailability

for uptake in either microbes or via plants into the food chain depends on pH, redox potential, and cation exchange capacity of soil, as well as adsorption to soil particles and interactions with soil microorganisms [8, 13]. The bioavailability further is strongly influenced by the presence of dissolved organic substances that may form metal complexes [14].

High heavy metal content in soils affects soil fertility as well as plant growth and renders large areas unsuitable for agricultural use. Thus, the remediation of heavy metal polluted soils is of high importance [6, 15]. Here, we discuss microbially assisted phytoremediation and also include an outlook to phytomining.

2 Potential of Phytoremediation Approaches

The remediation of heavy metal contaminated soils is one of the most challenging tasks for environmental engineering and most conventional remediation approaches do not provide satisfactory solutions [16, 17]. Conventional technologies for cleaning metal contaminated sites are mainly ex situ decontamination using physical and chemical methods. These ex situ techniques, such as soil washing, excavation, and thermal treatments, irreversibly affect soil functions, destroy biodiversity, and leave it biologically inactive [18, 19]. Furthermore, these remediation options are often costly, energy intensive, and site destructive [20]. The increasing awareness of public and governmental bodies provides an opportunity for plant-based bioremediation technologies.

The use of green plants to decontaminate heavy metal polluted sites, known as phytoremediation, is an in situ technology with considerable promise for removing metals from areas of low to moderate concentrations [21, 22]. It aims to use metal-accumulating plants to remove, transfer, or stabilize heavy metals at contaminated sites thus reducing the risk to the environment [23].

The idea of using plants that accumulate metals taken up from soil in harvestable biomass was introduced in 1983 and gained public exposure in 1990 [24]. Depending on the contaminants, the level of pollution, and the site conditions, phytoremediation includes five main plant-based technologies (Table 1) with three different mechanisms of action for cleaning up metal contaminated sites: phytoextraction, phytostabilization, and rhizofiltration [5, 25–28]. Among these, phytoextraction and phytostabilization are the most reliable for heavy metal removal from soils [29, 30].

The use of phytoremediation offers the benefits of being in situ, passive and solar-driven technology, and allows for site restoration, applicability to a wide range of sites, promoting future land use, and additionally opens the road to biorecovery of metals [31]. The main costs of phytoremediation are the site monitoring, the preconditioning of the contaminated soil, planting, potentially pest control, and harvest. It also contains the costs for disposal of contaminated biomass, mostly by controlled burning and ash deposition. The estimated costs for removal of contaminants from soils range from $25 to $100 per ton, depending on

Table 1 Phytoremediation types

Process	Description	Contaminant
Phytoextraction	Uptake of pollutants from soil and accumulation in harvestable plant biomass	Inorganic pollutants
Phytostabilization	Reduction of mobility and bioavailability of pollutants by plant roots in the rhizosphere	Inorganic pollutants
Rhizofiltration	Absorption and adsorption of pollutants by plant roots from aquatic environments	Inorganic/organic pollutants
Phytovolatilization	Removal of pollutants from contaminated environment and their release into air	Inorganic/organic pollutants
Phytodegradation	Degradation of pollutants by plants and associated microorganisms	Organic pollutants

site characterization and level of contamination [16, 25]. This cost-effective, green alternative may also be used at sites not readily available to other methods, reduces the exposure to secondary air- or water-borne wastes, and provides a vegetative ground cover for long-term stabilization and erosion prevention [32, 33]. The combination of bioenergy production and the recovery of heavy metals from metal-rich plant ash is possible [34]. Finally, remediation of contaminated sites by using green plants instead of machines or toxic chemicals is more attractive and more acceptable to the public than any other engineering-based approach.

However, some serious limitations of phytoremediation need to be considered. One of the greatest disadvantages is the time needed. Phytoremediation is generally slower than the more established, conventional soil remediation techniques such as excavation, incineration, or pump-and-treat systems. Several factors, including life cycles of plants, ordinary growing seasons, metal resistance of the crop used, as well as the level of contamination are influencing site cleanup [35]. In addition, site characteristics such as soil properties, mixed contamination, or climate may exert a strong influence. In addition, the use of plants does not allow a total removal of pollutants, because the lower the concentration of a respective pollutant, the slower the uptake becomes. This biological method is also limited in applicability to surface soils and limited by the bioavailability of the contaminant. Especially for cleaning up metal-contaminated sites, the solubility and bioavailability are of utmost importance [5, 36], and it requires further validation under field conditions in long-term experiments [6]. The use of chelating agents in order to enhance solubility of metals in soil, selection of adapted plant species, or addition of required nutrients or soil amendments might provide strategies to overcome disadvantages [16, 37].

Taken together, the success of the applied technology depends on two major components: choice of plants and soil conditions. Some plant species are well known to accumulate high metal loads in their biomass. Such metallophytes, however, often specifically concentrate one element, indicating limitations in remediation of sites with multiple contaminants. As a result, it is logical to consider crop plants as well that also have been evaluated for metal uptake in some cases. Decisive soil conditions such as homogeneous distribution of pollutants,

contamination with only one specific element, a good bioavailability of this contaminant, pH values between 4 and 7, and a good water-holding capacity of the contaminated soil are promising requirements [38].

There is one additional potential measure that has been underestimated thus far. The use of microbes with phytoremediation approaches might exert a positive influence on plant growth and soil function, which needs to be evaluated to the full before a final decision on feasibility can be made. This positive influence can lower the toxicity of metals in the plant or in the soil, increase the bioavailability of metals to achieve better uptake, reduce the wash-out with percolation water thus reducing the risk for ground and surface waters, or aid plant growth. The increase in biomass even may compensate for lower uptake per gram dry matter of harvested plant biomass. Thus, we discuss mechanisms of microbes, both bacteria and fungi, which are considered to be relevant for phytoremediation.

3 Plant-Based Methods for Bioremediation

3.1 Phytoextraction

Phytoextraction, also called phytoaccumulation, aims at removing inorganic pollutants, especially heavy metals, metalloids, and radionuclides, from contaminated subsurfaces through uptake by plants and accumulation in harvestable plant biomass [19]. Contributing factors for a successful extraction by plants are the extent and bioavailability of contaminants in soil and a plant's ability to tolerate and accumulate pollutants in high concentrations. For a successful metal extraction, the ideal plant should have some important characteristics: (1) rapid growth and high biomass production; (2) high tolerance to pollution and high accumulation of contaminants in aboveground biomass; (3) high root-to-shoot transfer of elements with a low binding capacity to root cell walls; (4) high bioconcentration factor and biological absorption coefficient (also referred to as BCF and BAC, respectively) higher than 1; (5) extended, well-branched, and deep root system; (6) native or easily adapting to the contaminated environment; and (7) simple agricultural management in the field [5, 38]. Unfortunately, even plant species suitable for phytoextraction do not combine all these required characteristics and poor soil conditions such as drought, moisture, and low fertility affect metal extraction. Suitable plants for phytoextraction are metal-accumulating crop species, especially within *Brassicaceae* and *Poaceae*, as well as highly productive tree species such as willow and poplar.

Most metal-tolerant plants are metal excluders. They restrict the transport and entry of metals into their aerial parts over a wide range of metal concentrations in the soil, but still contain high metal concentrations in their roots. Plants that actively accumulate metals in their upper plant tissues and generally reflect metal concentration in contaminated soil are called metal indicators [1, 2]. Some plant

Table 2 Main characteristics of continuous versus induced phytoextraction

Continuous phytoextraction	Chelate-assisted phytoextraction
Hyperaccumulator plants	Excluder, non-hyperaccumulator plants
Slow growth rates, low biomass production	High growth rates, high biomass production
Natural metal hyperaccumulation	Enhanced metal uptake by synthetic or natural chelators
Suitable for highly polluted soils	Suitable for low to moderate polluted soils
Most hyperaccumulators are metal specific	Multi-metal uptake
No environmental risk regarding leaching of metal chelates	Risk of percolation of anthropogenic metal chelates

species are able to accumulate specific metals to significant levels in their aboveground biomass. These hyperaccumulators can be used for a continuous phytoextraction because they accumulate metals at concentrations of more than 0.1 % or greater of their dry weight (Table 2; [39]). More than 400 species in 45 different botanical families can be classified as hyperaccumulator plants. Well-known plant families that contain species of hyperaccumulators are, for example, *Brassicaceae*, *Euphorbiaceae* and *Poaceae*. The hyperaccumulator plants, including *Thlaspi*, *Brassica*, *Apocynum*, *Paspalum*, and *Alyssum* [18], however, often are rather small with a low biomass production. There are, on the other hand, also some trees and shrubs that can accumulate elevated levels of specific metals without symptoms of phytotoxicity [5, 40].

In general, the feasibility of metal extraction from contaminated soil by plants is limited by the time required for cleanup, target metals in soil, depth of contamination, and suitable plant characteristics [41]. Thus, phytoremediation technique is applicable to decontaminate low to moderate metal-contaminated surface soils [38]. The effects of soil microbes discussed below may offer several beneficial traits.

3.2 Phytostabilization

During phytostabilization, metals are converted into inert immobilized species by absorption, adsorption, accumulation, precipitation, and physical stabilization within the root zone. The established vegetation cover provides the rhizosphere wherein metals precipitate. In this way, the plant action prevents metal leaching into groundwater [19]. Phytostabilization does not remove contaminants from soil, but aims at reducing the risk of further environmental degradation [42]. Desirable characteristics of plants selected for phytostabilization at a particular site include: (1) tolerance to high concentrations of metals of concern, (2) fast growth rates to establish ground cover and ability to develop an extended and abundant root system, (3) high retention capacity of contaminants in roots or rhizosphere to immobilize these contaminants and to prevent their spreading through the food chain, (4) low translocation of metals from root to shoots, (5) a high

bioconcentration factor, (6) relatively high transpiration rates to effectively dewater the soil, (7) low sustainment requirements and simple agricultural management, and (8) long-living and indigenous origin [43, 44]. For instance, suitable plants for phytostabilization are native species of perennial grasses, which are highly metal tolerant and adapted to local soil conditions. Additionally, a wide range of metal-tolerant shrubs and trees can be used for restoration of metal-contaminated sites [33, 45]. Typically, applied amendments are phosphate fertilizers, composted organic matter, liming agents, clay minerals, iron oxides, biosolids, or by-products from industrial processes [46]. The addition of soil amendments offers better starting conditions for the plants and may improve soil fertility [42, 47]. Here, microbially supported approaches may be used to substitute for amendments, at least partially.

3.3 Rhizofiltration

Rhizofiltration refers to using hydroponically cultivated roots or seedlings of terrestrial plants to absorb, concentrate, or precipitate metal pollutants from aqueous waste streams [48]. Mechanisms involved in metal removal by plant roots include extracellular precipitation, cell wall precipitation and surface adsorption, as well as intracellular uptake followed by compartmentalization and deposition within the vacuole [16, 19]. Suitable plants for rhizofiltration should combine the characteristics of: (1) high metal tolerance and high accumulation rates of target metals, (2) high translocation rates of metals, (3) high root biomass and large surface area, (4) easy handling and low maintenance costs, and (5) minimal secondary waste production. Fast-growing crop species including Indian mustard, sunflower, wild cabbage, tobacco, rye, and corn have an intrinsic ability to absorb and precipitate various heavy metals such as Pb, Mn, Cd, Ni, Cr, Cu, and Zn from aqueous solutions [16, 48]. At the same time, certain sunflower breeds seem to be promising candidates for rhizofiltration of radionuclides such as U, ^{137}Cs, and ^{90}Sr [27, 48]. Rhizofiltration seems to be most adaptable for large water volumes with a low level of contamination. The use of plant roots or seedlings provides an efficient and inexpensive solution to remove toxic metals from polluted waters and thus prevent hazardous risks to human health [16]. Removal of radionuclides from wastewaters may be particularly effective in combination with beneficial microorganisms [49].

4 Rhizosphere Interactions

Soil bacteria and mycorrhizal fungi have an impact on metal bioavailability and can either enhance or repress metal transfer from soil into harvestable plant biomass [39]. These interactions with direct contact or diffusion based interaction can

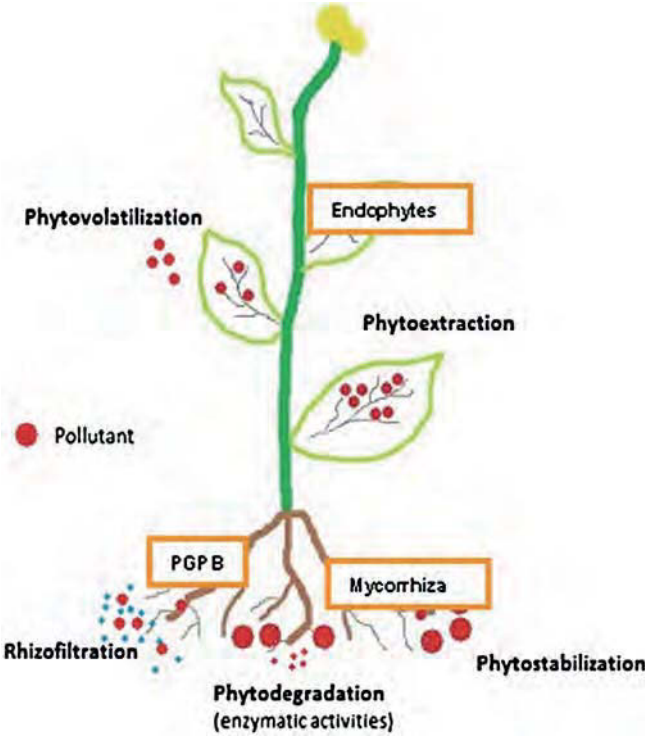

Fig. 1 Microbe–plant interactions in phytoremediation technologies (modified after [36])

be put into context with the general phytoremediation strategies (Fig. 1). A third possible microbe–plant interaction with impact on phytoremediation is the endophytic life style of bacteria or fungi within healthy plant tissues.

Specifically the mutually beneficial mycorrhizal symbiosis positively affects plant growth, biomass production, nutrient uptake, and production of growth-promoting hormones [50]. Within chemically assisted or induced phytoextraction, soil microbes may substitute the effects otherwise provided by application of chemical amendments to enhance the solubility and availability of metals in the soil [16, 51]. The secretion of natural chelating molecules such as phytosiderophores by plants [52] or metal reductases by roots [53] specifically can be complemented with soil bacteria that increase metal bioavailability and metal tolerance in plants [5].

But also alteration of physicochemical properties in the rhizosphere (e.g., pH and Eh) are prone to be dependent on microbial activity. Humic acids are formed by microbial degradation of dead organic matter. They become soluble at higher pH and make soil metals available for plants due to their characteristic carboxyl and phenol groups [54]. Furthermore, humic acids can reduce the mobility of various heavy metals in soil and limit percolation of solubilized metals into groundwater [55].

Phytohormones such as auxins have various positive effects on plants. For instance, auxins are involved in several cellular and physiological processes within the plant and can promote plant growth. Auxins are known to enhance both biomass production and root extension even in low concentrations, which may improve phytoextraction efficiency of metals by plants [5, 56]. Rhizosphere bacteria or fungi are well known to be able to synthesize auxins.

Most metal-accumulating plants are able to develop an extended root system with a high surface area to obtain essential nutrients for growth [16]. At the same time, they also have the ability to accumulate and tolerate elevated levels of nonessential metals. Usually, heavy metals in soil exist as ions and are taken up by plant roots via membrane transporter proteins. This active transport—against a chemical gradient—requires metabolic energy and allows for accumulation above the diffusion-driven adsorption to the apoplastic root surface. For most elements, numerous transporters exist in plants, each with specific properties with respect to transport rate, substrate affinity, and specificity [4, 57]. The storage of chelated metals in the vacuole or apoplast facilitates root sequestration [36].

The two major, heavy metal-binding compounds within plants are phytochelatins and metallothioneins [58]. Metallothioneins (MTs) are small cysteine-rich proteins found in animals and, more recently, in higher plants and bacteria. Typical for metallothioneins are their low molecular weight between 6–7 kDa and their high cysteine content necessary for coordination of specific metal ions in metal–thiolate clusters [59]. These low molecular weight metal-binding proteins are divided into three different classes, with class I being composed of animal MTs. MTs found in plants fall into class II, with wheat Ec protein and a number of MTs in different *Arabidopsis* ecotypes being described [2, 60, 61]. Within bacteria, metallohistins with high cysteine plus histidin contents can additionally be shown [62].

Phytochelatins (PCs) are a family of peptides that were first identified in yeast. They had been included into class III MTs. Whereas MTs are gene-encoded, PCs are enzymatically synthesized and induced by metals in most autotrophic plants, yeast, and some fungi. They are composed of only three amino acids, glutamine (Glu), cysteine (Cys), and glycine (Gly), and are structurally related to the tripeptide glutathione. The structure of these peptides is $(\gamma\text{-Glu-Cys})_nX$, where X is Gly, γ-alanine, serine, or glutamate, and n is in the range of 2–5, depending on the organism [63]. Biosynthesis of PCs can be induced by many metals, including Cd, Ni, Cu, Zn, Hg, Ag, Pb, and As, where Cd seems to be the strongest inducer [64]. PCs may play a role in the detoxification of metal ions by forming PC–metal complexes and thus regulate availability of metal ions in cells in order to prevent metal toxicity [65, 66].

4.1 Plant Growth Promoting Bacteria

Overviews of already published studies on benefits from plant–microbe interactions and their possible applications are given elsewhere [67–70]. The term *plant*

growth promoting bacteria (PGPB, or PGP rhizobacteria, PGPR) was introduced for those bacteria contributing to plant growth in both ways, directly or indirectly [71]. PGPB colonize root surfaces or thrive in the rhizosphere and affect plant development by nitrogen fixation, phosphate solubilization, or the production of phytohormones [69, 70], mostly auxins. Auxins are appropriate for phytoremediation, inasmuch as stimulation of germination, enhanced resistance to biotic or abiotic stress, and plant growth are controlled [69, 72].

Additionally, some PGPB are able to produce siderophores, iron chelators with a high Fe^{3+} affinity [73]. By binding to other metals, siderophores were found to promote plant growth on metalliferrous soils [74, 75]. Heavy metal resistant streptomycetes, Gram-positive aerobic soil bacteria, could be shown to enhance growth of *Vigna unguiculata* by siderophore production and nickel sequestration [76, 77] or preventing excess cadmium uptake in *Helianthus annuus* [78].

Enzymes, osmolytes, biosurfactants, nitric oxide, organic acids, and antibiotics produced by PGPB may contribute to the positive effects on plant performance [69]. However, additional studies should be undertaken to evaluate the sustainability and competition between PGPB and other soil microorganisms that need to be considered [80, 79].

4.2 Endophytes

Infection of plants without causing symptoms, in a harmless and mutualistic symbiosis, is performed by endophytic bacteria or fungi [81, 82]. Natural or genetically engineered endophytic bacteria were successfully used for phytoremediation studies [83–86]. These bacteria can improve a plants' capability of resisting pathogens, heavy metals, and herbivores. Additionally, enhancement in plant growth and supply with fixed nitrogen contribute to plant performance [87, 79]. In contrast to PGPB, endophytes live within healthy plant tissue, where stress and competition with other microbes are easier to overcome [79, 80]. Because fungal or bacterial cell walls are able to sequester substantial quantities of metals, an increase in metal loads of aboveground harvestable biomass seems possible.

One major point that needs consideration with respect to endophytes is that often, any organism isolated from surface-sterilized plant tissue is considered to be an endophyte. However, looking at the definition this is not true. It is very difficult to show that no contamination was remaining in the case of surface sterilization. Hence, it is mandatory that endophytes are reinfected, again growing without causing disease symptoms in the same compartment as before. Only if *in planta* growth can be re-established, should an organism be called an endophyte. However, once this is established, endophytes may well exert a positive effect, on metal sequestration. A mutually beneficial symbiosis may be assumed, however, here the correct denomination would be endophyte unless the beneficial traits to both partners have been clearly established (see [88, 89] and citations therein).

4.3 Mycorrhiza

Mycorrhizal interactions are mutually beneficial symbioses of higher plant roots and fungi [90]. In the environment, almost every plant undergoes mycorrhizal interaction with one or more fungal partners [91]. The plants profit from nutrient and water supplied through the fungus, which enhances plant growth and resistance against diseases [92]. In return, the plant supplies the fungus with glucose, sucrose, and other carbohydrates [90].

The two most common types of mycorrhiza are ecto- and endomycorrhiza, differentiated for lack or occurrence of root cell invasion by the fungus, respectively [91–92]. In both mycorrhiza types, the main fungal cell wall components chitin, cellulose derivatives, and melanin are able to bind and sequester heavy metals [94].

Ectomycorrhiza is ubiquitous in almost 10 % of plant families, especially ligneous plants, which form this root symbiosis with thousands of fungal species within over 200 genera [93, 95]. From the soil mycelium, which can transport nutrients towards the root from several hundred meters distance, ectomycorrhizal fungi form an outer mantle of hyphae covering the short root tips and develop to grow between the root rhizodermis cells, the Hartig' net [91]. Mainly, the benefit for phytoremediation is prevention of heavy metal toxicity [94]. For instance, accumulation of heavy metals has been found in cell wall layers, extramatrical hyphae, and the fungal mantle [94, 96, 97]. In pot experiments with copper and lead contaminated soil, *Betula pendula* has been shown to be protected from heavy metal stress due to colonization with ectomycorrhizal fungi. Although the mycorrhization rate decreased with higher heavy metal concentrations, the content of extracted copper and lead in *B. pendula* leaves was higher as compared to non-inoculated plants. Specifically young seedlings are found to profit from protection against metal stress [98]. A combined inoculation with ectomycorrhizal fungi and *Bacillus cereus* strains showed enhanced plant growth promotion for *Salix viminales* in contaminated soils and enhanced metallothionein production in the plant. Thus, a dual inoculation may be feasible for phytoextraction and phytostabilization [99].

Arbuscular mycorrhiza, the main type of endomycorrha, has been extensively investigated for phytoremediation [6, 90, 92, 94, 100]. Here, Glomeromycota fungi penetrate the root cortical cells [90]. The fungi are obligate biotrophs, not able to grow in the absence of green hosts for more than a few days, due to their inability to absorb carbohydrates [92, 100]. Different species, mostly of the genus *Glomus*, have been isolated from heavy metal contaminated soils. In plants inoculated with these isolates, heavy metals were found to be either more highly concentrated in plants, or were reduced due to mycorrhization [94]. Hence, there seems to be specific plant–fungal associations that need to be carefully combined and tested before field trials are performed, in order to establish a successful promotion of either phytoextraction or phytostabilization.

5 Metal Exclusion from Plant Uptake

The physiological properties of soil microbes not only allow for enhanced plant growth. It has been shown that specifically Gram-positive bacteria dominate at metalliferous sites [101]. One specific example featuring a field trial is the remediation effort at a former uranium mining site in Germany. Because field trials are still rare, these are of specific importance (Ebena and Kothe, [102]).

Different metal-resistance mechanisms of these bacteria may be useful for different remediation actions [103]. One useful property is that microbial biomass may, just like plant roots, immobilize metals in soil [104]. Bacterial and fungal cell walls have been investigated for metal sequestration from the water phase (e.g., [105]). Actively growing cells in soil are preferable over dead biomass, often used in (laboratory experiment) reports. The living microbes, in this case, need to be resistant against the prevailing metals in concentrations observed in the soil that is to be remediated (e.g., [106]).

In addition to the chemical properties of microbial cell walls, biomineralization (see, e.g., [107]) has been reported with heavy metal resistant soil bacteria, specifically streptomycetes. This group of soil bacteria has proven to be able to combine different mechanisms for heavy metal resistance [108], among them induction of metallothioneins and metallohistins [62]. Making use of different properties of metal-resistant soil bacteria thus may provide new approaches to phytoremediation. A thorough understanding of molecular mechanisms would aid such experimental approaches [3].

6 Metal Translocation into Plant Biomass

The chelation of metals in the root cells is followed by xylem loading and translocation into the shoot which involves two main processes: (1) movement from root symplast into xylem apoplast, and (2) enhanced volume flux through the xylem. The transport from root endodermis into the root xylem is achieved by membrane transporter proteins. The process of xylem loading with metals is energized by a negative membrane potential generated by proton pumping ATP-ases [109, 110]. In the xylem, metals are chelated by organic acids (e.g., histidine, citrate, and malate), nicotianamine, thiol-rich peptides (e.g., glutathione, phytochelatins), or cysteine-rich metallothioneins [16, 111]. This complexation prevents metal immobilization in the xylem and enables movement into the shoot. Unfortunately, for most metals, it is still unclear which transporter proteins are involved in their export to the root xylem and to which chelators they are bound during transfer to above-ground parts.

Epidermal and subepidermal tissues, including leaf trichomes, are sites of metal sequestration in plant tissues. Leaf epidermal cells are preferred compartments, because they allow for removal with leaf litter [112, 113]. Metal-tolerant plants are

able to control and change the solution concentration of free metal ions in their cellular compartments and thus are able to survive at highly contaminated sites.

7 Potentials for Phytomining

The obvious technique for phytomining is to use hyperaccumulator plants for removal of metal ions from the growth substrate. Worldwide, about 450 plant species in different taxa, ranging from annual herbs to perennials have been identified as hyperaccumulators. Approximately two-thirds of these species are known to hyperaccumulate Ni [1]. Only 30 plant species are known to accumulate Cd, Co, Cu, or Zn in large amounts, and there are no known Pb hyperaccumulators yet [114, 115]. Hyperaccumulators show an exceptionally high metal tolerance, efficient root-to-shoot translocation, and high uptake rates of metals to achieve this remarkable accumulation of toxic soil metals. Their hypertolerance to certain metals may result from vacuolar compartmentalization and metal chelation [26]. Boyd et al. [116] have demonstrated that high concentrations of Ni in leaves of the hyperaccumulator plant *Thlaspi montanum* var. *montanum* can protect plants against herbivores and pathogens [116, 117].

The use of hyperaccumulators to remove heavy metals from contaminated soil was first suggested by (Chaney [118]) and 10 years later by McGrath et al. [119]. The concept of phytomining involves the recovery of marketable amounts of metals from incineration ashes. The first studies on Ni phytomining were carried out by Nicks and Chambers in 1994, by using the Californian hyperaccumulator *Streptanthus polygaloides* to extract Ni from serpentine soils. The Ni concentration in this soil was about 0.35 %, well below the economic concentration for direct mining [120, 121]. The second field trials in phytomining for nickel were carried out in Tuscany, Italy, by Robinson et al. [122] using the Ni hyperaccumulator *Alyssum bertolonii*. They could show that *A. bertolonii* can be used to phytomine Ni commercially, and that the use of fertilizers can increase Ni content in plants [122]. The third recorded phytomining field trial for Ni used the high-biomass Ni hyperaccumulator *Berkheya coddii*, an asteraceous perennial plant that can grow to a height of about 2 m. Under controlled field conditions, a dry biomass of 22 t/ha could be obtained after moderate fertilization within one growth period, the highest reported yield [122].

Suitable plants for phytomining should have the characteristics of: (1) high biomass production, (2) easy to grow from seeds, (3) perennial, (4) hardy and adapted to local climatic conditions, and (5) resistance to herbivores and pathogens [123]. Several strategies might be useful to make phytomining a viable technique for the recovery of metals from contaminated plant biomass. These include the use of high-biomass hyperaccumulators with a high metal content, or the use of fertilizers to increase plant biomass and metal yields, where a high metal yield is to

be preferred over high biomass. The use of microbially aided phytoaccumulation has not been explored this far.

Other strategies discussed include amendment with chelating agents, such as EDTA/EGTA, or bioengineering of hyperaccumulators to increase biomass [124]. Phytomining with high-biomass hyperaccumulators would offer the possibility of exploiting ores or metalliferrous soils that are uneconomic for conventional mining techniques. The extracted metals are essentially free of sulfur; their smelting requires less energy than sulfidic ores [34] and does not contribute to acid rain. They often contain more than one metal and have a lower density than conventional ores, and thus require comparatively small space for storage [34]. This green and emerging technology could provide an alternative to open-cast mining of low-grade ores, but its commercialization depends on the metal content of the harvested biomass and the world price of the target metal. At the same time, the economic feasibility of phytomining is limited by its low efficiency with respect to land use and time. Research is required to overcome these potential limitations to make phytomining a successful commercial technique in recovering metals from contaminated soil by plants [12, 125]. These approaches might be even further stimulated by considering endophytic bacteria and fungi (for reviews see [126–128]). However, thus far this route to enhance the phytomining potential of hyperaccumulating plants has not been pursued.

8 Case Study

For a proof-of-principle, a case study is included here. This is within the former uranium mining area in Eastern Thuringia and Western Saxony, Germany, where mining during German Democratic Republic times produced 210,000 tons of uranium for the USSR weapon industry. The mining operations were stopped in 1990 with the reunification of Germany, and remediation of the vast area was started [129]. The size of the mining-related contamination made a multiple-step approach necessary. The mine was closed, the shafts and galleys sealed to prevent easy flow of mine water to the surface, and flooded. The heaps were removed into the former open-pit mine in a structured way by putting in the most acid-generating, sulfidic material at the lowest point and the most neutralizing at the top. The flooding of the mine re-establishes anaerobic conditions preventing further oxidation of the material and thus limiting the future production of acid mine drainage. The former heap sites were recontoured using allochthonous material. In only a few cases heaps were retained and covered. Tailings were stabilized and prepared for dry cover. Finally, acid mine drainage influenced waters have been removed to water treatment plants. All in all, the size of the operation was tremendous and the sum of €6.5 billion was needed to come to this technical solution, performed by the WISMUT GmbH [129]. However, this huge effort still leaves environmental problems unsolved, as could be expected, given the size of the operation.

Fig. 2 Test field site at the time of adding different soil amendments before establishing the site in 2004, and in 2010 after seven planting seasons

One of these remaining areas with problematic environmental influences is the former leaching heap Gessen near Ronneburg, Thuringia, Germany. Here, low-grade ore had been leached resulting in significant problems with acid mine drainage waters influencing the heap base at points where an initial loam base had leaked. At the time of removing the heap material, the base material was removed to a depth of approximately 3–6 m and replaced by a cover of 40–100 cm of new material [130–132]. The area was sown with a mixture of grasses and clover. However, the acidic and heavy metal rich water by capillary rise led to metal contamination of the surface substrate, and plant growth has been limited in this area., The Friedrich Schiller University in Jena established a test field site in this area in 2004 (Fig. 2) where the feasibility of phytoremediation is tested [133]. The setting clearly covers the above-mentioned preconditions for phytoremediation, namely a spatially heterogeneous, comparatively low contamination of a vast area, where geotechnical and engineering solutions are not (or not further) feasible [134].

Several lysimeters were installed to monitor input into groundwater, and sunflowers were sown for five years, inoculated with soil bacteria isolated from the site, and mycorrhizal fungi. In addition, it was tested whether a soil amendment could enhance plant growth ([135, 136]; see also Fig. 2). Thus, 5 cm of topsoil or 5 cm of compost were plowed in with the upper 30 cm of substrate. It was not meant as a cover in which plants could grow, but rather a moderate addition of nutrients to the nutrient-deprived substrate. At the same time, an inoculation was achieved with the compost. This was supposed to be important because we had seen only limited numbers of bacteria in the deprived soil material at the site. Indeed, 9 years after the addition of the amendments, an effect is still observed, even if the added nutrients have already been consumed a long time ago, and the mixed material is acidic and now also metal contaminated [104]. However, establishing a microbial community able to survive the harsh conditions led to soil formation and the beginning processes of pedogenesis enhance plant growth (Fig. 2). Hence, the initial concept of first increasing soil microbiology to see a secondary positive effect on plant performance has proven to be successful.

Had the initial purpose been to neutralize the soil by addition of calcareous material, the prevailing soil microbiology would have been even further diminished. This has been known for a long time and has been seen, for example, after forest soil neutralization as an acid rain counter-measure. Indeed, the loss of trees, at least initially, is strongly enhanced, mainly because the ectomycorrhizal fungi stabilizing this ecosystem are adapted to lower pH and cannot survive the sudden pH increase. The loss of their symbionts is even more detrimental to the trees as compared to the slow decrease resulting from acid rain (see, e.g., [137]).

A similar situation adhering to the same principle of destroying soil microbiology would have been observed had we chosen to add fertilizer to our plots. In this case, the plants would likely not have responded as strongly to the microbial community. Although fertilization is a short-term effect, enhancing microbial activity leads to a longer lasting improvement of soil functions associated with soil microbiology and hence seems preferable, even if associated with a lower initial biomass production. This effect was tested at the field site using different plants and monitoring soil respiration throughout all different planting regimes (Fig. 3). Indeed, soil respiration was influenced by a change of planting regime, as has also been observed with agricultural soils.

The application of microbes, certainly, depends on the sustainability of the added microbes within the autochthonous community. Thus, isolation of indigenous strains, cultivation, and re-application seem advisable, rather than providing a one-for-all “cure strain”. This concept has been tested at the test field site and indeed, a sustainable effect could be observed [138].

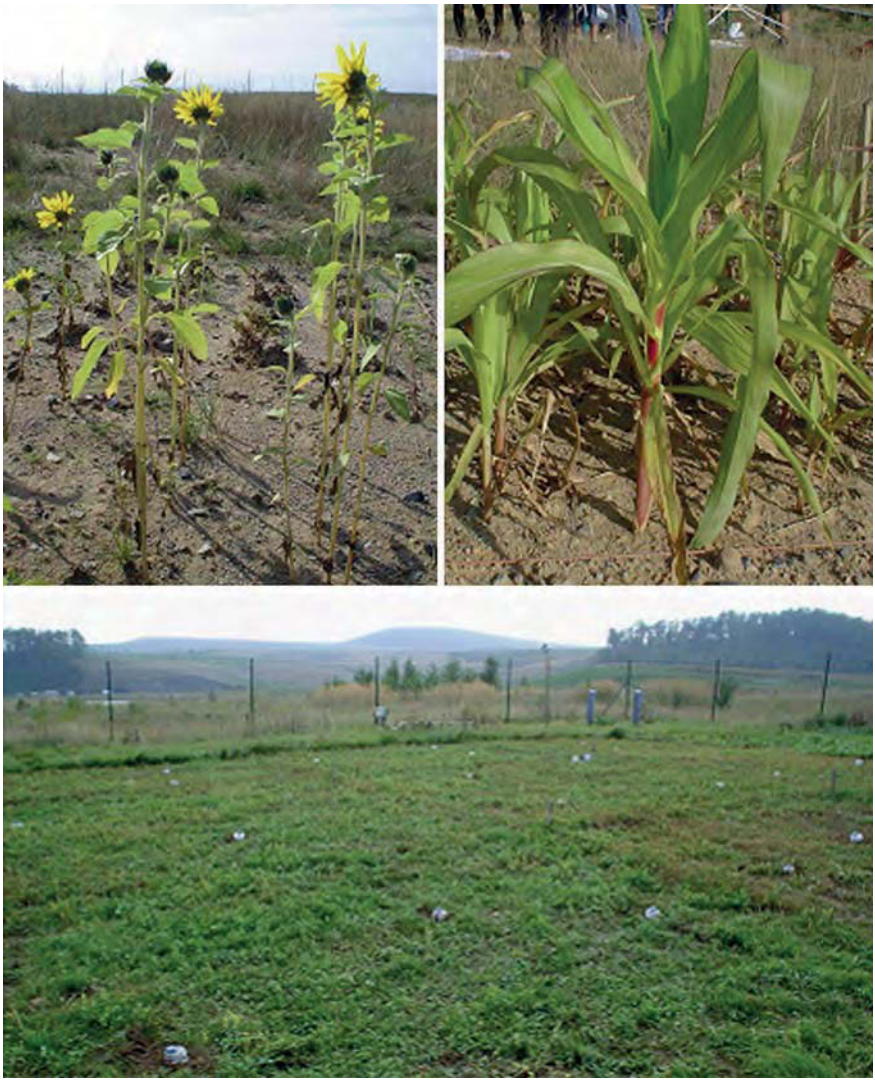

Fig. 3 Planting regimes at the test field site resulted in limited biomass production of sunflower (*Helianthus annuus*) and corn (*Zea mays*). Soil respiration (*lower image*) was obtained in situ

9 Conclusions

The increased metal mining and industrial use of metals lead to both local high contamination and vast areas of heterogeneous, low to medium metal contamination. Whereas for local, highly contaminated areas, geotechnology approaches provide good solutions, the remediation of large landscapes with lower, but still detrimental metal content in the soil may well be prone to undergo biotechnological

remediation. Here, we discussed the potential of phytoremediation assisted by microbial processes for such use.

The results of our review show that microbes may support different tasks in bioremediation applications, from phytostabilization to phytomining. Phytostabilization does not promise to remove the contaminant, but instead provides a solution for establishing a ground cover with the help of soil microbes. The microbes may immobilize metals such that neither uptake into food chains nor excess plant toxicity occurs. Thus, the beneficial effects of a ground cover, with enhanced evapotranspiration and protection from wind and water erosion can be provided. Such a revegetation on usually nutrient-deprived soils strongly benefits from the plant growth promotion of either rhizobacteria or mycorrhizal fungal associations with plant roots. At the same time, sequestration by soil microbes or biomineralization limits contamination of groundwater needed for the drinking water supply in many places.

Another application of phytostabilization is the use of contaminated land for farming, not for production of food or human consumption crops, but for production of bioenergy plants. This allows for sustainable energy production without direct competition with agriculture for food crops. However, to be used in bioethanol or bioenergy production, metal loads of harvested plant biomass needs to be below legislation thresholds.

Soil bacteria, endophytes, and mycorrhizal fungi may, on the other hand, also help in tipping the balance in favor of phytoextraction. The mobilizing activities of PGPB or soil fungi may be especially helpful in achieving high metal uptake into plant-harvestable biomass. Here, specifically the excretion of chelators and acidification potential of physiologically active soil microbes may be useful for geobiotechnology. Even in phytomining, these activities are worth considering. Here, the metal sequestration within plant tissue apoplast at bacterial or fungal cell walls and the compartmentalization, for example, in fungal vacuoles, might further increase both metal tolerance and metal accumulation of plants.

All in all, microbially assisted phytoremediation is only beginning to be explored and field trials are especially urgently needed to evaluate the feasibility and stability of geobiotechnological approaches in metal bioremediation.

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References

1. McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232:207–214
2. Memon AR, Schröder P (2009) Implications of metal accumulation mechanisms to phytoremediation. Env Sci Pollut Res 16:162–175
3. Haferburg G, Kothe E (2010) Metallomics: lessons for metalliferous soil remediation. Appl Microbiol Biotechnol 87:1271–1280

4. Hassan Z, Aarts MGM (2011) Opportunities and feasibilities for biotechnological improvement of Zn, Cd or Ni tolerance and accumulation in plants. Env Exp Bot 72:53–63
5. Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land. A review. Env Chem Lett 8:1–17
6. Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364
7. Sheoran V, Sheoran AS, Poonia P (2011) Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: A review. Crit Rev Env Sci Technol 41:168–214
8. Gardea-Torresdey JL, Peralta-Videa JR, de la Rosa G, Parsons JG (2005) Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. Coord Chem Rev 249:1797–1810
9. Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51:730–750
10. Bruins MR, Kapil S, Oehme FW (2000) Microbial resistance to metals in the environment. Ecotox Env Safety 45:198–207
11. Schmidt A, Haferburg G, Sineriz M, Merten D, Büchel G, Kothe E (2005) Heavy metal resistance mechanisms in actinobacteria for survival in AMD contaminated soils. Chem Erde Geochem 65:131–144
12. Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? and what makes them so interesting? Plant Sci 180:169–181
13. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Ann Rev Plant Physiol Plant Mol Biol 49:643–668
14. Kordel W, Dassenakis M, Lintelmann J, Padberg S (1997) The importance of natural organic material for environmental processes in waters and soils. Pure Appl Chem 69:1571–1600
15. Yanez L, Ortiz D, Calderon J, Batres L, Carrizales L, Mejia J, Martinez L, Garcia-Nieto E, Diaz-Barriga F (2002) Overview of human health and chemical mixtures: problems facing developing countries. Env Health Perspect 110:901–909
16. Salt DE, Blaylock M, Kumar N, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation—a novel strategy for the removal of toxic metals from the environment using plants. Bio-Technol 13:468–474
17. Mulligan CN, Yong RN, Gibbs BF (2001) Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng Geol 60:193–207
18. Arthur EL, Rice PJ, Anderson TA, Baladi SM, Henderson KLD, Coats JR (2005) Phytoremediation—an overview. Critical Rev Plant Sci 24:109–122
19. Padmavathiamma PK, Li LY (2007) Phytoremediation technology: hyper-accumulation metals in plants. Water Air Soil Pollut 184:105–126
20. Mulligan CN, Yong RN, Gibbs BF (1999) On the use of biosurfactants for the removal of heavy metals from oil-contaminated soil. Env Progress 18:50–54
21. Cunningham SD, Berti WR, Huang JW (1995) Phytoremediation of contaminated soils. Trends Biotechnol 13:393–397
22. Cunningham SD, Shann JR, Crowley DE, Anderson TA (1997) Phytoremediation of contaminated water and soil. Am Chem Soc 664:2–17
23. Raskin I, Salt D, Krämer U, Schulman R (1998) Phytoremediation: green and clean. Acta Horticult (ISHS) 457:329–332
24. Raskin I, Kumar PBAN, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290
25. Cunningham SD, Anderson TA, Schwab AP, Hsu FC (1996) Phytoremediation of soils contaminated with organic pollutants. Adv Agron 56:55–114
26. Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8:279–284
27. Dushenkov S, Kapulnik Y, Blaylock M, Sorochisky B, Raskin I, Ensley B (1997) Phytoremediation: a novel approach to an old problem. Studies Env Sci 66:563–572
28. Dushenkov S, Vasudev D, Kapulnik Y, Gleba D, Fleisher D, Ting KC, Ensley B (1997) Removal of uranium from water using terrestrial plants. Env Sci Technol 31:3468–3474

29. Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Env Sci Technol 29:1232–1238
30. Burken JG, Schnoor JL (1997) Uptake and metabolism of atrazine by poplar trees. Env Sci Technol 31:1399–1406
31. Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resour Conserv Recycl 11:41–49
32. Vangronsveld J, Colpaert JV, van Tichelen KK (1996) Reclamation of a bare industrial area contaminated by non-ferrous metals: physico-chemical and biological evaluation of the durability of soil treatment and revegetation. Env Pollut 94:131–140
33. Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Env Int 29:529–540
34. Anderson CWN, Brooks RR, Chiarucci A, LaCoste CJ, Leblanc M, Robinson BH, Simcock R, Stewart RB (1999) Phytomining for nickel, thallium and gold. J Geochem Expl 67:407–415
35. Boyd V (1996) Pint-sized plants pack a punch in fight against heavy metals. Env Prot 7:38–39
36. Pilon-Smits E (2005) Phytoremediation. Ann Rev Plant Biol 56:15–39
37. Huang JWW, Chen JJ, Berti WR, Cunningham SD (1997) Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. Env Sci Technol 31:800–805
38. Ernst WHO (2005) Phytoextraction of mine wastes—options and impossibilities. Chem Erde Geochem 65:29–42
39. Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresource Technol 77:229–236
40. Boyd RS, Jaffre T, Odom JW (1999) Variation in nickel content in the nickel-hyperaccumulating shrub *Psychotria douarrei* (Rubiaceae) from New Caledonia. Biotropica 31:403–410
41. van Nevel L, Mertens J, Oorts K, Verheyen K (2007) Phytoextraction of metals from soils: how far from practice? Env Pollut 150:34–40
42. Mendez MO, Maier RM (2008) Phytostabilization of mine tailings in arid and semiarid environments—an emerging remediation technology. Env Health Perspect 116:278–283
43. Kucharski R, Sas-Nowosielska A, Malkowski E, Japenga J, Kuperberg JM, Pogrzeba M, Krzyzak J (2005) The use of indigenous plant species and calcium phosphate for the stabilization of highly metal-polluted sites in southern Poland. Plant Soil 273:291–305
44. Yoon J, Cao XD, Zhou QX, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Env 368:456–464
45. Geebelen W, Adriano DC, van der Lelie D, Mench M, Carleer R, Clijsters H, Vangronsveld J (2003) Selected bioavailability assays to test the efficacy of amendment-induced immobilization of lead in soils. Plant Soil 249:217–228
46. Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, van der Lelie D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Env Sci Pollut Res 16:765–794
47. Pichtel JR, Dick WA, Sutton P (1994) Comparison of amendments and management practices for long-term reclamation of abandoned mine lands. J Env Qual 23:766–772
48. Dushenkov S, Kumar PBAN, Motto H, Raskin I (1995) Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. Env Sci Technol 29:1239–1245
49. Macaskie LE (1991) The application of biotechnology to the treatment of wastes produced from the nuclear fuel cycle: Biodegradation and bioaccumulation as a means of treating radionuclide-containing streams. Crit Rev Biotechnol 11:41–112
50. Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139–153

51. Kaschl A, Romheld V, Chen Y (2002) Cadmium binding by fractions of dissolved organic matter and humic substances from municipal solid waste compost. J Env Qual 31:1885–1892
52. Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226
53. Welch RM, Norvell WA, Schäfer SC, Shaff JE, Kochian LV (1993) Induction of iron(III) and copper(II) reduction in pea (*Pisum sativum* L.) roots by Fe and Cu status—does the root-cell plasmalemma Fe(III)-chelate reductase perform a general role in regulating cation uptake. Planta 190:555–561
54. Lagier T, Feuillade G, Matejka G (2000) Interactions between copper and organic macromolecules: determination of conditional complexation constants. Agronomie 20:537–546
55. Halim M, Conte P, Piccolo A (2003) Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. Chemosphere 52:265–275
56. Zhou J-M, Dang Z, Chen N-C, Xu S-G, Xie Z-Y (2007) Enhanced phytoextraction of heavy metal contaminated soil by chelating agents and auxin indole-3-acetic acid. Huan Jing Ke Xue 28:2085–2088
57. Abedin MJ, Feldmann J, Meharg AA (2002) Uptake kinetics of arsenic species in rice plants. Plant Physiol 128:1120–1128
58. Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Env Qual 31:109–120
59. Kagi JH, Kojima Y (1979) Nomenclature of metallothionein: a proposal. Experientia Suppl 34:141–142
60. Lane B, Kajioka R, Kennedy T (1987) The wheat-germ Ec protein is a zinc-containing metallothionein. Biochem Cell Biol-Biochim Biol Cellulaire 65:1001–1005
61. Murphy A, Zhou JM, Goldsbrough PB, Taiz L (1997) Purification and immunological identification of metallothioneins 1 and 2 from *Arabidopsis thaliana*. Plant Physiol 113:1293–1301
62. Schmidt A, Hagen M, Schütze E, Schmidt A, Kothe E (2010) *In silico* prediction of potential metallothioneins and metallohistins in actinobacteria. J Basic Microbiol 50:562–569
63. Rauser WE (1995) Phytochelatins and related peptides (structure, biosynthesis, and function). Plant Physiol 109:1141–1149
64. Grill E, Löffler S, Winnacker E-L, Zenk MH (1989) Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific γ-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). Proc Nat Acad Sci U S A 86:6838–6842
65. Thumann J, Grill E, Winnacker E-L, Zenk MH (1991) Reactivation of metal-requiring apoenzymes by phytochelatin-metal complexes. FEBS Lett 284:66–69
66. Yang X-E, Jin X-F, Feng Y, Islam E (2005) Molecular mechanisms and genetic basis of heavy metal tolerance/hyperaccumulation in plants. J Integr Plant Biol 47:1025–1035
67. Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412
68. Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009) Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. J Appl Microbiol 107:1687–1696
69. Ma Y, Prasad MN, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258
70. Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Gen Mol Biol 35:1044–1051
71. Kloepper JW, Schroth MN (1979) Plant-growth promoting rhizobacteria—evidence that the mode of action involves root microflora interactions. Phytopathology 69:1020–1024
72. Teale WD, Paponov IA, Palme K (2006) Auxin in action: signalling, transport and the control of plant growth and development. Nat Rev Mol Cell Biol 7:847–859
73. Neilands JB (1984) Methodology of siderophores. Struct Bonding 58:1–24

74. Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant-growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885–886
75. Schalk IJ, Hannauer M, Braud A (2011) New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol 13:2844–2854
76. Dimkpa C, Svatos A, Merten D, Büchel G, Kothe E (2008) Hydroxamate siderophores produced by *Streptomyces acidiscabies* E13 bind nickel and promote growth in cowpea (*Vigna unguiculata* L.) under nickel stress. Can J Microbiol 54:163–172
77. Dimkpa CO, Svatos A, Dabrowska P, Schmidt A, Boland W, Kothe E (2008) Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in *Streptomyces spp*. Chemosphere 74:19–25
78. Dimkpa C, Weinand T, Asch F (2009) Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Env 32:1682–1694
79. Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333
80. Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77:153–160
81. Misaghi IJ, Donndelinger CR (1990) Endophytic bacteria in symptom-free cotton plants. Phytopathology 80:808–811
82. Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M, van der Lelie D (2002) Endophytic bacteria and their potential applications. Crit Rev Plant Sci 21:583–606
83. Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert JV, Vangronsveld J, van der Lelie D (2004) Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nature Biotechnol 22:583–588
84. Germaine KJ, Keogh E, Ryan D, Dowling DN (2009) Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiol Lett 296:226–234
85. Germaine KJ, Liu X, Cabellos GG, Hogan JP, Ryan D, Dowling DN (2006) Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol 57:302–310
86. Weyens N, CroesS Dupae J, Newman L, van der Lelie D, Carleer R, Vangronsveld J (2010) Endophytic bacteria improve phytoremediation of Ni and TCE co-contamination. Env Pollut 158:2422–2427
87. Selosse MA, Baudoin E, Vandenkoornhuyse P (2004) Symbiotic microorganisms, a key for ecological success and protection of plants. Crit Rev Biol 327:639–648
88. Kothe E, Dudeja SS (2011) Editorial. J Basic Microbiol 51:4
89. Dudeja SS, Giri R, Saini R, Suneja-Madan P, Kothe E (2012) Interaction of endophytic microbes with legumes. J Basic Microbiol 52:248–260
90. Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Ann Rev Microbiol 59:19–42
91. Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic Press, San Diego
92. Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nature Comm 1:48
93. Rinaldi AC, Comandini O, Kuyper TW (2008) Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Div 33:1–45
94. Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207
95. Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363
96. Wilkins DA (1991) The influence of sheathing (ecto-) mycorrhizas of trees on the uptake and toxicity of metals. Agricult Ecosyst Env 35:245–260
97. Turnau K, Kottke I, Dexheimer J, Botton B (1994) Element distribution in mycelium of *Pisolithus arrhizus* treated with cadmium dust. Annals Bot 74:137–142

98. Bojarczuk K, Kieliszewska-Rokicka B (2010) Effect of ectomycorrhiza on Cu and Pb accumulation in leaves and roots of silver birch (*Betula pendula* Roth.) seedlings grown in metal-contaminated soil. Water Air Soil Pollut 207:227–240
99. Hrynkiewicz K, Dabrowska G, Baum C, Niedojadlo K, Leinweber P (2012) Interactive and single effects of ectomycorrhiza formation and *Bacillus cereus* on metallothionein MT1 expression and phytoextraction of Cd and Zn by willows. Water Air Soil Pollut 223:957–968
100. Bonfante P (2010) Plant–fungal interactions in mycorrhizas., Encyclopedia of Life SciencesWiley, London
101. Narula N, Reinicke M, Haferburg G, Kothe E, Behl RK (2012) Plant-microbe interaction in heavy metal-contaminated soils. In: Kothe E, Varma A (eds) Bio-geo interactions in heavy metal-contaminated soils. Springer, Heidelberg, pp 143–162
102. Ebenå G, Kothe E (2006) Bioleaching and microbial mineral degradation. In: Varma A (ed) Microbes: health and environment, vol 3. IK International Publishing, New Delhi, pp 389–405
103. Haferburg G, Kothe E (2012) Biogeosciences in heavy metal-contaminated soils. In: Kothe E, Varma A (eds) Bio-geo interactions in heavy metal-contaminated soils. Springer, Heidelberg, pp 17–34
104. Schindler F, Gube M, Kothe E (2012) Bioremediation and heavy metal uptake: microbial appraoches at field scale. In: Koth E, Varma A (eds) Bio-geo interactions in heavy metal-contaminated soils. Springer, Heidelberg, pp 365–384
105. Merten D, Geletneky J, Bergmann H, Haferburg G, Kothe E, Büchel G (2005) Rare earth element patterns: a tool for remediation of acid mine drainage. Chem Erde Geochem 65S1:97–114
106. Schmidt A, Haferburg G, Schmidt A, Lischke U, Merten D, Gherghel F, Büchel G, Kothe E (2009) Heavy metal resistance to the extreme: *Streptomyces* strains from a former uranium mining area. Chem Erde Geochem 69:35–44
107. Haferburg G, Klöß G, Schmitz W, Kothe E (2008) "Ni-struvite"—a new biomineral formed by a nickel resistant *Streptomyces acidiscabies*. Chemosphere 72:517–523
108. Kothe E, Dimkpa CO, Haferburg G, Schmidt A, Schmidt A, Schütze E (2010) Streptomycete heavy metal resistance: extracellular and intracellular mechanisms. In: Sherameti I, Varma A (eds) Soil heavy metals. Springer, Heidelberg, pp 225–236
109. Pitman M (1972) Uptake and transport of ions in barley seedlings II. Evidence for two active stages in transport to the shoot. Aus J Biol Sci 25:243–258
110. Boer AH, Prins HBA, Zanstra PE (1983) Bi-phasic composition of trans-root electrical potential in roots of *Plantago* species: involvement of spatially separated electrogenic pumps. Planta 157:259–266
111. Stephan UW, Schmidke I, Stephan VW, Scholz G (1996) The nicotianamine molecule is made-to-measure for complexation of metal micronutrients in plants. Biometals 9:84–90
112. Vögeli-Lange R, Wagner GJ (1990) Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves: implication of a transport function for cadmium-binding peptides. Plant Physiol 92:1086–1093
113. Kupper H, Zhao FJ, McGrath SP (1999) Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 119:305–311
114. Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126
115. Prasad MNV (2005) Nickelophilous plants and their significance in phytotechnologies. Braz J Plant Physiol 17:113–128
116. Boyd RS, Shaw JJ, Martens SN (1994) Nickel hyperaccumulation defends *Streptanthus polygaloides* (Brassicaceae) against pathogens. Am J Bot 81:294–300
117. Boyd RS, Martens SN (1994) Nickel hyperaccumulated by *Thlaspi montanum* var. montanum is acutely toxic to an insect herbivore. Oikos 70:21–25

118. Chaney RL (1983) Plant uptake of inorganic waste. In: Parr JE, Marsh PB, Kla JM (eds) Land treatment of hazardous waste. Noyes Data Corp, Park Ridge, pp 50–76
119. McGrath SP, Sidoli CMD, Baker AJM, Reeves RD (1993) The potential for the use of metal-accumulating plants for the in situ decontamination of metal-polluted soils. In: Eijsackers HJP, Hamers T (eds) Integrated soil and sediment research : a basis for proper prediction. Kluwer, Dordrecht, The Netherlands, pp 673–676
120. Nicks L, Chambers MF (1998) A pioneering study of the potential of phytomining for nickel. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals. CAB International, Wallingford, pp 312–325
121. Nicks L, Chambers MF (1995) Farming for metals. Mining Environ Meeting 199:15–18
122. Robinson BH, Brooks RR, Howes AW, Kirkman JH, Gregg PEH (1997) The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. J Geochem Expl 60:115–126
123. Brooks RR, Robinson BH, Howes AW, Chiarucci A (2001) An evaluation of *Berkheya coddii* Roessler and *Alyssum bertolonii* Desv. for phytoremediation and phytomining of nickel. S Afr J Sci 97:558–560
124. Brooks RR, Robinson BH (1998) The potential use of hyperaccumulators and other plants for phytomining. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CAB International, Wallingford, pp 327–356
125. Robinson BH, Banuelos G, Conesa HM, Evangelou MWH, Schulin R (2009) The phytomanagement of trace elements in soil. Critical Rev Plant Sci 28:240–266
126. Newman LA, Reynolds CM (2005) Bacteria and phytoremediation: new uses for endophytic bacteria in plants. Trends Biotechnol 23:6–8
127. Mastretta C, Barac T, Vangronsveld J, Newman L, Taghavi S, van der Lelie D (2006) Endophytic bacteria and their potential application to improve the phytoremediation of contaminated environments. Biotechnol Genet Eng Rev 23:175–207
128. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9
129. Gatzweiler R, Jakubick AT, Meyer J, Paul M, Schreyer J (2003) Flooding of the WISMUT mines - learning by doing or applying a comprehensive systematic approach ? In: Merkel B, Planer-Friedrich B, Wolkersdorfer C (eds) Uranium in the aquatic environment. Springer, Heildelberg, pp 745–754
130. Grawunder A, Lonschinski M, Merten D, Büchel G (2009) Distribution and bonding of residual contamination in glacial sediments at the former uranium mining leaching heap of Gessen/Thuringia, Germany. Chem Erde - Geochem 69S2:5–19
131. Lonschinski M, Knöller K, Merten D, Büchel G (2011) Flow dynamics of groundwater and soil water in the former heap Gessenhalde at the uranium mining area of Ronneburg: a stable isotope approach. Hydrol Process 25:861–872
132. Senitz S, Gaupp R, Büchel G (2009) Hochaufgelöste dreidimensionale Rekonstruktion eines quartären Talaquifers in einem Teilbereich des Gessentals bei Ronneburg (ehemaliges ostthüringisches Uranerzbergbaurevier) durch die Kombination von Geländedaten und geostatistischer Schätzung. Zeitschr dtsch Ges Geowiss 160:367–389
133. Kothe E, Bergmann H, Büchel G (2005) Molecular mechanisms in bio-geo-interactions: from a case study to general mechanisms. Chem Erde Geochem 65S1:7–27
134. Willscher S, Mirgorodsky D, Jablonski L, Ollivier D, Merten D, Büchel G, Wittig J, Werner P (2013) Field scale phytoremediation experiments on a heavy metal and uranium contaminated site, and further utilization of the plant residues. Hydrometallurgy F:46–53
135. Büchel G, Bergmann H, Ebenå G, Kothe E (2005) Geomicrobiology in remediation of mine waste. Chem Erde 65S1:1–5
136. Mirgorodsky D, Ollivier D, Merten D, Bergmann H, Büchel G, Willscher S, Wittig J, Jablonski L, Werner P (2010) Radiation protection of radioactively contaminated large areas by phytoremediation and subsequent utilization of the contaminated plant residues. J Nucl Power 55:774–778

137. Demchik MC, Sharpe WE (2001) Forest floor plant response to lime and fertilizer before and after partial cutting of a northern red oak stand on an extremely acidic soil in Pennsylvania, USA. Forest Evol Manag 144:239–244
138. Reinicke M, Schindler G, Roth M, Kothe E (2013) Multi-metal bioremediation by microbial assisted phytoremediation. In: Actinobacteria. Application in bioremediation and production of industrial enzymes (Amorosos MJ, Benimeli CS, Cuozzo SA, Eds.). CRC, Boca Raton, USA, pp 87–105.

3.2 Manuscript II

Phytoremediation using microbially mediated metal accumulation in *Sorghum bicolor*

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Phytoremediation using microbially mediated metal accumulation in *Sorghum bicolor*

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Abstract Reclaiming land that has been anthropogenically contaminated with multiple heavy metal elements, e.g., during mining operations, is a growing challenge worldwide. The use of phytoremediation has been discussed with varying success. Here, we show that a careful examination of options of microbial determination of plant performance is a key element in providing a multielement remediation option for such landscapes. We used both (a) mycorrhiza with *Rhizophagus irregularis* and (b) bacterial amendments with *Streptomyces acidiscabies* E13 and *Streptomyces tendae* F4 to mediate plant-promoting and metal-accumulating properties to *Sorghum bicolor*. In pot experiments, the effects on plant growth and metal uptake were scored, and in a field trial at a former uranium leaching heap site near Ronneburg, Germany, we could show the efficacy under field conditions. Different metals could be extracted at the same time, with varying microbial inoculation and soil amendment scenarios possible when a certain metal is the focus of interest. Especially, manganese was extracted at very high levels which might be useful even for phytomining approaches.

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Keywords Phytoremediation · Heavy metals · *Sorghum bicolor* · Field trial · Pot experiments · *Rhizophagus* · *Streptomyces*

Introduction

Heavy metal and metalloid soil pollution through anthropogenic activities such as mining and smelting operations, burning of fossil fuels, applications of insecticides or fertilizers, and waste disposal are an increasing problem (Khan 2005; Yoon et al. 2006). High metal loads affect soil functions including soil structure and its productivity and may lead to contamination of ground- and surface waters (Ali et al. 2013; Vamerali et al. 2009). Decontamination of metal-polluted soils (Baker et al. 1994; Raskin et al. 1997) may be achieved by conventional remediation approaches like ex situ application of physicochemical methods; however, these techniques are both destructive and costly (Arthur et al. 2005; Saraswat and Rai 2009). Alternatively, bioremediation provides a sustainable and cost-efficient solution with phytoremediation aiming to apply metal accumulation in harvestable plant biomass (phytoextraction) with subsequent burning and ash deposition or to decrease metal mobility and toxicity (phytostabilization) (Brunetti et al. 2011; Dushenkov et al. 1997).

Phytoremediation strategies offer several benefits: they can be performed in situ and at low cost, prevent destroying soil structure and function, provide a vegetative ground cover abating erosion, and even are permissible for future land use and biorecovery of valuable metals (McGrath et al. 2001; Yang et al. 2005). Limitations that have been encountered are connected to soil properties, level of contamination, and bioavailability of pollutants (Pilon-Smits 2005). To overcome such limitations, organic or inorganic amendments have been

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applied which, however, may result in nonpredictable results (Raskin et al. 1997). An alternative strategy is to modulate phytoremediation by addressing the microbial activities in the rhizosphere (Bolan et al. 2014; Sullivan et al. 2013).

Soil bacteria and mycorrhizal fungi can alter physicochemical properties in the rhizosphere and affect plant growth, thus changing metal uptake, e.g., by secretion of phytohormones (Zhuang et al. 2007b), production of chelators and siderophores (Dimkpa et al. 2009a; Raskin et al. 1997), acidification, and biomineralization (Abou-Shanab et al. 2008; Lasat 2002). It has been shown that specifically Gram-positive bacteria such as streptomycetes are ubiquitous in metalliferous soils where they thrive due to specific metal resistance traits aiding, in turn, plant growth (Abbas and Edwards 1989; Dimkpa et al. 2008, 2009a; Haferburg and Kothe 2007; Schmidt et al. 2005, 2009). These interactions between metal-tolerant soil microorganisms and plant roots play a significant role in remediation of heavy metals. Their beneficial effects on plant growth through nitrogen fixation, solubilization of phosphate, or acting as biocontrol agents (Ahemad and Kibret 2014) are well-studied features of plant-associated microorganisms with which they improve efficiency of phytoremediation. Already improved growth, increased metal bioavailability, and protection of plants against phytotoxic metal effects are among the desired characteristics of microbial bioinoculants for improved phytoremediation (Lasat 2002; Weyens et al. 2009).

The potential of heavy metal-resistant bacteria for enhancing the growth of host plants in contaminated soil has been reported (Nogueira et al. 2007; Sessitsch et al. 2013) For instance, *Streptomyces mirabilis* has been found to improve biomass productivity of *Sorghum bicolor* in metal-contaminated soil (Schütze et al. 2014). Fast growing crop plants, like *S. bicolor*, offer several advantages for phytoremediation processes because of its high biomass production, stress tolerance, and metal accumulation potential (Ciura et al. 2005; Epelde et al. 2009; Marchiol et al. 2007; Murillo et al. 1999; Zhuang et al. 2009). In the work presented here, we evaluated the application of two metal-resistant *Streptomyces* strains, isolated from a former uranium mining site and the arbuscular mycorrhizal fungus *Rhizophagus irregularis* for microbially assisted phytoremediation approaches. The study investigated the impact of microbial amendment on plant performance and metal extraction by *S. bicolor* and examined metal mobility in contaminated soil in pot experiments and with field trials.

Material and methods

Site description and soil analysis

Pot and field experiments were carried out using contaminated soil material from the test site Gessenwiese installed by the University of Jena in 2004 on the basement of the former uranium leaching heap Gessenhalde near Ronneburg in Eastern Thuringia, Germany. Between 1952 and 1990, low-grade uranium ores were leached by irrigation with acid mine drainage (AMD) waters or diluted sulfuric acid (Büchel et al. 2005; Neagoe et al. 2005). After uranium mining operations were stopped in 1990, remediation started for restoration of this contaminated site. However, the drainage waters within this experimental site (Gessenwiese) still show high concentrations of heavy metals resulting in a spatially heterogeneous, but comparatively low, multimetal contamination (Schindler et al. 2012).

Soil samples were air-dried and sieved to a grain size up to 2 mm for determination of soil pH and total digestions and for sequential extractions. Soil pH was measured after shaking a 1:4 suspension for 1 h, left to settle for 24 h, and measured using pH330 (WTW). The same solution was used to determine electrical conductivity (EC; TetraCon 325 and LF320, WTW). Total heavy metal contents were determined using a pressure digestion system (DAS 30, PicoTrace). The bioavailable fraction of soil elements was determined following sequential extraction (Zeien and Brümmer 1989). The mobile fraction (F1) was extracted with 1 M NH_4NO_3 (p.a., Merck; compare Grawunder et al. 2009). Element contents were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES; 725 ES, Varian) and inductively coupled plasma-mass spectrometry (ICP-MS, X-Series II, Thermo Fisher Scientific) in triplicates. The metal concentrations for total contents and bioavailable fractions are added as values before planting (t_0 at day 0) in the respective experiments, where t_0 was compared to soil concentrations after planting and inoculation. The sandy silt (53.93 % silt, 46.07 % sand) showed a cation exchange capacity of 9.07 mol/kg with a water content of 5.55 to 17.57 % and very low values for carbon, nitrogen, and sulfur as main nutrients (below detection limit for N and S, 1.01 to 1.17 % C).

Preparation of microbial inocula

The two multiresistant strains *Streptomyces acidiscabies* E13 and *Streptomyces tendae* F4 isolated from the former uranium mining site near Ronneburg, Germany (Amoroso et al. 2000), were used as bacterial inoculum. These strains are known to tolerate high concentrations of toxic metals and further for their plant growth promotion traits (Dimkpa et al. 2008; Schmidt et al. 2005). To prepare the bacterial inoculum for pot and field experiments, strains were cultivated in fermenters (7-L BIOSTAT B-DCUII, Sartorius Stedim Systems, or 300L Braun Biotech International). *S. acidiscabies* E13 was grown in medium 3 (glucose monohydrate, 5 g/l; soluble starch, 25 g/l; casein-peptone, 10 g/l; yeast extract, 5 g/l; $(NH_4)_2SO_4$, 1.5 g/l; KH_2PO_4, 1.5 g/l; trace element solution, 1 ml [$ZnCl_2$ 40 mg/l, $FeCl_3 \cdot 6\ H_2O$ 200 mg/l, $CuCl_3 \cdot 6\ H_2O$

10 mg/l, $MnCl_2 \cdot 4\ H_2O$ 10 mg/l, $Na_2B_4O_7$ 10 mg/l, $(NH_4)_2Mo_7O_{24} \cdot 6\ H_2O$ 10 mg/l, pH 7.0]) and *S. tendae* F4 was grown in medium 2 (replacing the C sources of medium 3 with glucose monohydrate, 30 g/l; casein-peptone, 10 g/l; cornsteep [Roquette]). Precultures for inoculating the fermenters were grown in the same media with additional 5 g/l $CaCO_3$. Fermentation conditions were 25 °C, 500 rpm, pO_2>20 %, aeration 2 slpm, and pH>6 controlled with 10 % NaOH (only for *S. tendae* F4). After 42 h of growth, mycelium was harvested by centrifugation (6000 rpm, 15 min, Avanti J-20 XP, Beckman) or separation (300–400 l/h, CSA8, Westfalia) and resuspended in tap water. Dead biomass was obtained by autoclaving. The arbuscular mycorrhizal inoculum was obtained as expanded clay containing spores of *R. irregularis* (Biofa AG, Münsingen, Germany) with 100 spores per gram.

Pot experiments

Pot experiments were carried out from May to October, 2012, on *S. bicolor* plants grown in a greenhouse (Thüringer Landesanstalt für Landwirtschaft, Jena). The setup consisted of 40 polyethylene pots (12×12×16 cm) filled with 2.5 kg contaminated soil from the test site. Each pot was sowed with 23 seeds of *S. bicolor*. After germination, seedlings were thinned to 12 plants per pot. The experimental design included four treatments: a negative control (unamended, C), amended with *Streptomyces* strains (S), amended with mycorrhizal fungus (M), and amended with a mixture of the two streptomycetes and the mycorrhizal fungus (MS). All treatments were carried out in five replicates.

Microbial inoculation was performed by mixing 20 ml of bacterial suspension and/or 4 g of *R. irregularis* granulate at the time of seeding. The pots were arranged in a randomized pattern and randomly rearranged every 4 days. Plants grew with natural day/night rhythm at ambient temperature between 15 and 30 °C. All plants were irrigated daily with distilled water. Aboveground biomass was harvested at 3 and 6 months after planting.

Field experiment

The field experiment was carried out from May to September, 2013, on the test site Gessenwiese (50° 51′ 27″ N and 12° 08′ 82″ E) in the former uranium mining district Ronneburg, Germany (Büchel et al. 2005). *S. bicolor* was cultivated in two different plots of 12× 12 m each, one of which had been amended with 5 cm of calcareous topsoil in 2004 (topsoil plot), while the second plot was left unamended (control plot). *Sorghum* plants were subjected to three experimental treatments in three replicates at each plot: unamended control (C), inoculated with mycorrhizal *R. irregularis* (M), and inoculated with a mixture of mycorrhiza and streptomycetes (MS). For microbial inoculation, a volume of 20 l of bacterial suspension and/or granulate of *R. irregularis* as recommended were applied per subplot (Neagoe et al. 2014; Schindler et al. 2012). Harvest occurred after 17 weeks.

Plant analyses

After harvesting, plant shoots were thoroughly washed with deionized water and oven-dried at 40 °C until constant weight to determine shoot dry weight. Plants were then ground to a fine powder using an ultracentrifugal mill (ZM100, Retsch). Up to 200 mg of plant material was weighted and digested with 5 ml HNO_3 (65 %, supra, Merck) in a microwave pressure system (Mars 5 XPRESS, CEM, Germany). The digested samples were transferred into 25 ml flasks filled up with ultrapure water (PureLab Plus, USF Elga) and analyzed for heavy metals by ICP-OES (725 ES, Varian) and ICP-MS (X-Series II, Thermo Fisher Scientific) in triplicates. The precision and accuracy of the ICP-MS and ICP-OES measurements were proven by analyzing standard reference material SPS-SW2 (Spectrapure Standards AS) and NIST 1643e (NIST) and by measuring multielement standard solution (500 mg/l Ca, K, Mg, Bernd Kraft) each in dilution 1:5 (*v*/*v*) and comparison to the certified values. Typical precision for triplicate measurements was ≤2 % for ICP-MS and ≤5 % for ICP-OES.

Statistical analyses

All statistical analyses were performed with R 3.0.3. The data were analyzed for variance (ANOVA) with a confidence level of 95 %. Significant differences between treatment means were confirmed by Tukey's test or, for nonparametric data, by Kruskal-Wallis test (P<0.05). Means and standard deviations were calculated using Microsoft Excel 2007 (Microsoft Corporation) for Windows 7.

Results

Plant performance on contaminated substrate under glasshouse conditions

After 3 and 6 months of plant growth, the influence of microbial inoculation on biomass production of *S. bicolor* was evaluated by measuring shoot weight (Fig. 1). The biomass productivity of inoculated plants that were treated with both mycorrhiza and streptomycetes showed a slight, albeit statistically significant increase after 3 months.

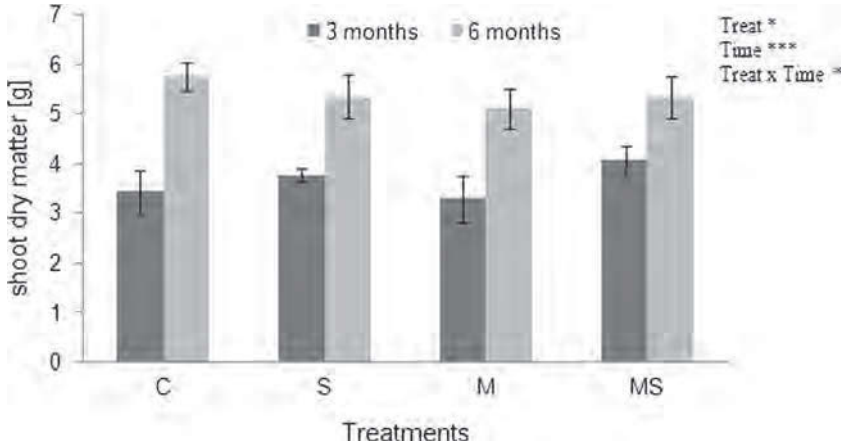

Fig. 1 Effects of treatments on shoot dry weight of *Sorghum bicolor* per pot after 3 and 6 months of growth in potting substrate. Values represent means±SD (n=5). Two-way ANOVA was performed to determine the effects of treatments and time. Significance levels of time, treatments (Treat), and the interaction treat×time are shown: *P<0.05; **P<0.01; ***P<0.001. *C* control treatment without inoculation, *S* amended with streptomycetes, *M* amended with mycorrhiza, *MS* amended with mycorrhiza and streptomycetes

The uptake of metals into shoots of *Sorghum* plants (Table 1) showed significant differences between treatments. Highest concentrations of Al, Co, and Ni were observed in the shoots of noninoculated *Sorghum* plants, while highest amounts of Cd, Mn, Sr, and Zn were found in plants with microbial amendment after 3 months.

After 6 months, *Sorghum* plants without microbial inoculation accumulated significantly higher levels of Al, Co, Mn, Ni, and Zn, while a significant contribution of bacterial and mycorrhizal inoculation could be observed for Cd and Sr uptake. The low bioavailability of U resulted in very low concentrations (0.01 without standard deviation) and was not further considered.

Treatment effects on metal availability and contents in the potting substrate

Both total metal contents and bioavailability were examined in order to evaluate the potential of microbially assisted phytoremediation under controlled conditions. The substrate showed multimetal contamination with high Al bioavailability at pH 4.4 to 4.6 and an electrical conductivity of 439± 12 μS cm^{-1}. Soil bacteria and mycorrhizal fungi can change soil pH and, hence, alter bioavailability. Additional metal tolerance mechanisms including chelator or siderophore production may lead to changes in metal transfer from soil into plant biomass. Thus, the changes in bioavailable metal contents

Table 1 Metal concentrations in shoots of *Sorghum bicolor* grown in greenhouse pots

Growth time	Treatments	Metal concentration in shoots [mg kg^{-1}]							
		Al	Cd	Co	Mn	Ni	Sr	U	Zn
3 months	Control	99.6±31.9	1.19±0.13	0.98±0.17	278±39	43.9±7.9	8.0±0.6	0.01±0.00	12.0±0.9
	Streptomyces	75.5±24.9	1.19±0.20	0.81±0.26	289±22	31.7±14.2	7.7±0.6	0.01±0.00	12.1±1.5
	Mycorrhiza	86.9±18.6	1.34±0.15	0.68±0.07	320±36	24.5±1.6	9.4±0.8	0.01±0.00	10.7±2.1
	Mycorrhiza+*Streptomyces*	58.9±11.1	1.16±0.11	0.56±0.04	281±14	21.4±2.7	8.1±0.9	0.01±0.00	10.6±1.2
6 months	Control	41.9±9.2	0.57±0.06	0.59±0.20	271±27	25.0±7.8	7.5±0.5	0.01±0.00	17.6±3.3
	Streptomyces	25.7±5.7	0.55±0.08	0.44±0.20	252±19	20.1±7.4	7.1±0.8	0.01±0.00	14.2±2.9
	Mycorrhiza	25.5±9.4	0.54±0.13	0.42±0.05	264±18	15.5±2.4	7.4±0.5	0.01±0.00	14.6±2.6
	Mycorrhiza+*Streptomyces*	25.2±4.3	0.62±0.12	0.40±0.14	263±24	16.2±2.4	7.8±0.3	0.01±0.00	13.3±1.7
ANOVA	Treatment	**	n.s.	**	n.s.	***	*	*	n.s.
	Time	***	***	***	**	***	***	*	***
	Treatment×time	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.

n.s. nonsignificant at the P<0.05 level

*P<0.05; **P<0.01; ***P<0.001

within the soil after the plant growth season were checked with respect to the different microbial treatments. For most metals, a decrease in mobile fraction was seen after the pot experiment, most prominent for Al, while a slight mobilization of Co and Mn had occurred for both inoculated and noninoculated pots after 6 months. In contrast, Cd showed very low mobilization for all treatments (Table 2). The inoculation did show a statistically significant effect on reduction of mobile Al and Ni contents.

Biomass production and metal uptake in the field trial

S. bicolor was grown in both field substrates which featured pH 5.2 to 5.4 and conductivity of 276±60 μS cm^{-1} for the topsoil field site and pH 4.4 to 5.2 and 249±99 μS cm^{-1} for the control soil plot. The microbial amendments of either mycorrhiza or a mixture of mycorrhiza and streptomycetes were applied to evaluate microbial impact on plant growth. This was following the results of the pot experiments in which either mycorrhiza alone or, in most parameters, combined streptomycete and mycorrhiza application had induced changes in metal bioavailability.

As the field site had been amended in 2004 with 5 to 10 cm topsoil to allow for better plant performance, this effect was evaluated in addition to microbial inoculation. Generally, an effect on biomass production by adding low amounts of topsoil in 2004 was not observed. In addition, for plants grown on the topsoil plot, microbial inoculation did not enhance biomass production (Fig. 2). In contrast, in the nonamended control soil, aboveground biomass was significantly increased by combined inoculation with mycorrhiza and streptomycetes. Thus, an effect of topsoil addition was seen, albeit only with the help of microbial amendments. While microbial addition could help plant growth on the unamended control soil, the topsoil addition had been sufficient—potentially even by adding the soil microbial community—to support plant growth in a way that made additional microbial inoculation superfluous.

In line with the lack of a measurable effect of inoculation on the topsoil-treated field site, microbial inoculation had no significant effect ($P<0.05$) on general metal accumulation in *S. bicolor* shoots (Table 3). This was found for both substrates. In a more detailed analysis, the topsoil field-grown plants showed higher levels of U and Zn in inoculated subplots. However, the combined application of mycorrhiza and streptomycetes decreased the uptake of Ni into shoots. On the unamended control soil, Co and Mn were accumulated in high amounts into shoot biomass of inoculated *Sorghum*, while the concentration of Ni was lowest in the shoots of plants treated with mycorrhiza.

To test the effects of planting and inoculation on the respective substrate, metal mobility was scored by sequential extraction before and after planting (Table 4). In the topsoil plot, neither plant growth nor inoculation induced visible changes in metal mobility recorded at the end of the growing season for most metals. However, there was a significant increase in Sr in the mobile fraction, while U availability was slightly reduced. Only few significant changes in bioavailable metal concentrations were detected for the control soil, with increases in Al and U and decreases in Co and Mn after plant growth.

Discussion

Phytoextraction of heavy metals by using crop species with high biomass production is a promising approach to remediate

Table 2 Alteration of soil metal bioavailability in pots after plant growth

Soil metal content [mg kg^{-1}]		Al	Cd	Co	Mn	Ni	Sr	Zn
Before planting	Total soil content	57820±300	0.52±0.03	14.7±0.1	477±7	54±1.4	96.6±2.3	75.4±2.1
	Bioavailable	51.3±0.3	0.09±0.00	0.41±0.02	46.6±0.8	5.4±0.1	3.3±0.0	1.91±0.03
3 months	Control	46.1±1.8	0.09±0.01	0.28±0.03	39±2	5.5±0.2	3.5±0.1	1.7±0.1
	Streptomyces	47.4±1.1	0.08±0.01	0.30±0.00	39±2	5.5±0.2	3.4±0.0	1.7±0.1
	Mycorrhiza	47.5±2.4	0.08±0.00	0.26±0.02	36±1	5.6±0.2	3.5±0.1	1.7±0.0
	Mycorrhiza+*Streptomyces*	48.5±1.2	0.08±0.00	0.29±0.02	37±1	5.5±0.3	3.4±0.1	1.6±0.1
6 months	Control	35.6±1.3	0.08±0.01	0.58±0.16	49±8	4.9±0.3	3.2±0.2	1.5±0.0
	Streptomyces	34.9±0.2	0.08±0.01	0.54±0.02	50±1	4.9±0.0	3.2±0.0	1.7±0.0
	Mycorrhiza	34.3±0.4	0.08±0.00	0.54±0.13	49±8	4.8±0.1	3.3±0.2	1.5±0.1
	Mycorrhiza+*Streptomyces*	30.1±2.7	0.09±0.01	0.54±0.03	49±3	4.3±0.2	3.0±0.2	1.5±0.1
Statistical significance	Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Time	***	n.s.	***	***	***	***	***
	Treatment×time	**	n.s.	n.s.	n.s.	*	n.s.	n.s.

n.s. nonsignificant at the $P<0.05$ level

*$P<0.05$; **$P<0.01$; ***$P<0.001$

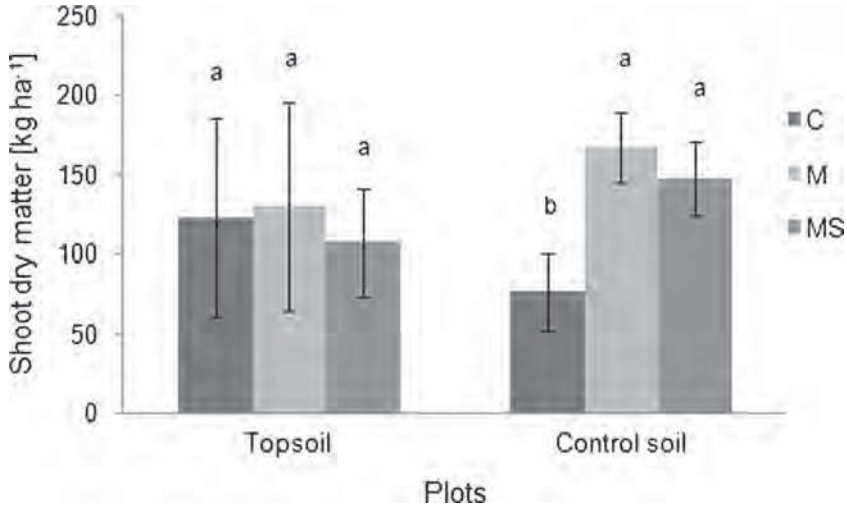

Fig. 2 Effects of treatments on shoot dry weight of *Sorghum bicolor* grown in two different field substrates. Values represent means±SD (n=9). One-way ANOVA was performed for each field substrate. *Means with different letters* are significantly different (P<0.05) according to Tukey's test. *C* control treatment without inoculation, *S* amended with streptomycetes, *M* amended with mycorrhiza, *MS* amended with mycorrhiza and streptomycetes

low to moderately contaminated soils (Ernst 2005). In our study, we evaluated the role of microbial inoculation on metal extraction and uptake by *S. bicolor* grown on multimetal-contaminated soil. Besides metal accumulation capacity, plant biomass production was measured to define a phytoextraction potential for this particular plant species known to tolerate heavy metals including Zn, Cu, Cd, Ni, and Pb (Hernández-Allica et al. 2008; Zhao et al. 2003; Zhuang et al. 2007a). We did not consider root concentrations which are often included in phytoextraction calculations. The crop harvestable biomass exclusively consists of the aerial part, and thus, roots may not be considered for phytoextraction or phytomining as long as the root is not harvested (which would be the case for, e.g., potato or beets).

The diffusive metal translocation in soil and root apoplast reflects the bioavailability of a given metal, making it essential to analyze the bioavailable fraction. The impact of soil microbes on bioavailability is an essential part influencing plant uptake. Soil bacteria and mycorrhizal fungi facilitate an increase in soil metal mobility (Zhuang et al. 2007b) and can significantly promote heavy metal uptake by plants (Rojas-Tapias et al. 2012; Usman and Mohamed 2009).

Metal-resistant streptomycetes were applied to enhance plant performance. These Gram-positive, aerobic soil bacteria have been found to promote plant growth on metalliferrous soils (Dimkpa et al. 2008, 2009b). The effects of streptomycetes on plant development can be triggered by various mechanisms including phosphate solubilization, production of phytohormones, and siderophore excretion (Dimkpa et al. 2009a; Langella et al. 2014). Additionally, metal-resistant arbuscular mycorrhizal fungi have been extensively investigated for application in soil remediation (Griffioen 1994; Khan 2005; Khan et al. 2000; Turnau et al. 2001). They can support growth of host plants in metal-contaminated environments by enhanced uptake of nutrients and water and by modification of metal toxicity via complexation or precipitation (Ernst 2005; Gaur and Adholeya 2004; Wang et al. 2007). In mycorrhizal plants, toxic elements were found to be either more highly concentrated or reduced through fungal metal-binding processes within the rhizosphere (Toler et al. 2005; Usman and Mohamed 2009).

A dual inoculation with arbuscular mycorrhizal fungi and rhizospheric bacteria, specifically streptomycetes, showed enhanced plant biomass productivity and increased levels of mycorrhization (Abdel-Fattah and Mohamedin 2000). In contrast, antagonistic interactions between AM fungi and actinomycetes have also been reported (Adriano-Anaya et al. 2006; Ames et al. 1984; Schreiner and Koide 1993), underlining the

Table 3 Metal concentrations in shoots of *Sorghum bicolor* grown in the field trial

Metal concentration [mg kg^{-1}]		Al	Cd	Co	Mn	Ni	Sr	U	Zn
Topsoil	Control	54.4±5.7	2.8±1.3	1.3±0.8	397±152	21.0±13	7.4±0.6	0.02±0.00	21.1±7.1
	Mycorrhiza	57.4±11.4	2.2±1.7	1.0±0.2	308±15	13.1±2.9	7.4±1.5	0.02±0.01	24.5±12.9
	Mycorrhiza+*Streptomyces*	53.1±16	2.6±1.6	1.4±0.2	385±125	12.3±2.2	7.6±0.6	0.03±0.00	30.1±15.8
Control soil	Control	164±32	3.8±1.3	1.6±0.6	494±126	28.2±3.1	7.6±1.1	0.10±0.02	35.1±16.1
	Mycorrhiza	126±4.6	2.0±0.1	0.8±0.3	303±92	18.3±1.8	8.2±0.8	0.07±0.01	13.3±0.8
	Mycorrhiza+*Streptomyces*	144±24.5	2.1±0.1	3.2±0.8	798±134	29.0±2.4	6.2±0.5	0.08±0.02	14.5±0.2

Table 4 Alteration of soil metal bioavailability in the field after plant growth

Metal concentration [mg kg^{-1}]		Al	Cd	Co	Mn	Ni	Sr	U	Zn
Topsoil	Total soil content	50178±632	0.8±0.1	15.7±1.1	664±63	53.6±2.8	106±3	5.8±0.2	79.4±2.1
	Bioavailable	6.5±3.3	0.17±0.02	0.8±0.55	119±27	8.9±2.3	6.1±0.5	0.004±0.001	3.6±0.9
	Control	17.8±8.8	0.25±0.08	0.33±0.12	92.1±15.5	12.9±5.1	6.3±0.1	0.003±0.00	4.6±1.2
	Mycorrhiza	9.4±5.3	0.19±0.04	0.69±0.42	92.6±24.6	12.0±6.2	6.8±0.3	0.004±0.00	4.3±1.9
	Mycorrhiza+*Streptomyces*	8.3±5.3	0.18±0.04	0.60±0.65	90.8±28.5	10.4±2.8	7.0±0.4	0.003±0.00	4.0±1.0
ANOVA	Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Time	n.s.	n.s.	n.s.	n.s.	n.s.	*	***	n.s.
	Treatment×time	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Control soil	Total soil content	54181±1508	0.72±0.07	17.0±2.0	663±179	53.7±5.3	106±2	6.5±0.2	70.4±1.2
	Bioavailable	33.3±17.2	0.19±0.05	0.87±0.35	87.6±14.9	9.8±2.3	2.9±0.2	0.023±0.001	2.4±0.9
	Control	47.6±4.8	0.20±0.01	0.24±0.10	57.8±4.0	10.6±1.4	3.2±0.3	0.04±0.01	2.2±0.5
	Mycorrhiza	58.1±14.2	0.21±0.06	0.26±0.12	60.5±17.1	10.7±4.3	3.1±0.2	0.04±0.02	2.6±1.2
	Mycorrhiza+*Streptomyces*	74.6±27.5	0.22±0.06	0.44±0.35	72.0±21.8	11.7±3.1	3.0±0.1	0.06±0.03	2.8±1.2
Statistical significance	Treatment	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Time	**	n.s.	**	**	n.s.	n.s.	*	n.s.
	Treatment×time	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. nonsignificant at the $P<0.05$ level

*$P<0.05$; **$P<0.01$; ***$P<0.001$

necessity to first score the microbial interactions in pot experiments.

The potential of metal removal of a given plant species is mainly influenced by its metal accumulation capacity and biomass productivity (Lasat 2002; Zhuang et al. 2007a). In our experiment, microbial inoculation could partially enhance shoot metal concentration of *Sorghum* plants under greenhouse conditions, while due to high variability, the effect was less clear under field conditions. These differences in metal uptake between small-scale and open environment, large-scale field trials may be caused by different physiological states of the plants and heterogeneous soil conditions.

The bioconcentration factor (BCF; metal concentration in harvested shoots/soil content in mobile fraction F1) is one of the most important variables for a successful phytoextraction process (McGrath and Zhao 2003). *Sorghum* plants showed relatively high BCF values for Cd and Zn (Table 5). Shoot metal concentrations, including BCF values, decreased during growth except for Zn (compare Epelde et al. 2009).

Since only a small fraction of heavy metals is bioavailable for plant uptake, it is necessary to follow metal mobility in soil (Violante et al. 2010). Besides physicochemical properties like soil pH, redox potential, or metal speciation, which strongly influence bioavailability of heavy metals, soil microorganisms can significantly promote metal solubility and mobilization in the soil through acidification or by producing chelators (Marques et al. 2013; Sheng et al. 2012). The microbial inoculation had a significant impact on reduction of bioavailable soil fractions of Al and Ni after 6 months of plant growth under controlled conditions (see also Schütze et al. 2014).

Table 5 Bioconcentration factors for metal accumulation into *Sorghum* shoots (shoot metal concentration/soil content in mobile fraction F1)

Treatments		Al	Cd	Co	Mn	Ni	Sr	U	Zn
Pot experiment	Control	1.9	13.4	2.4	6.0	8.1	2.4	0.8	6.3
	Streptomyces	1.5	13.5	2.0	6.2	5.9	2.3	0.7	6.4
	Mycorrhiza	1.7	15.2	1.7	6.9	4.5	2.8	0.8	5.6
	Mycorrhiza+*Streptomyces*	1.1	13.1	1.4	6.0	4.0	2.4	0.6	5.6
Field experiment on topsoil plot	Control	20.0	16.5	2.9	3.4	2.3	1.2	6.4	6.8
	Mycorrhiza	8.8	14.2	1.6	2.9	1.6	1.3	5.2	7.1
	Mycorrhiza+*Streptomyces*	8.9	15.8	2.3	3.0	1.4	1.2	5.8	7.9
Field experiment on control soil plot	Control	1082.1	27.5	3.6	6.8	3.3	2.4	18.0	26.7
	Mycorrhiza	4.5	11.9	0.8	3.6	2.3	2.8	3.3	7.1
	Mycorrhiza+*Streptomyces*	4.3	10.9	4.2	8.5	3.1	2.2	3.7	5.9

Thus, our study support the use of *Sorghum* for phytoextraction specifically for Cd and Co, while microbial inoculation can lead to higher plant survival by minimizing the toxic effects of other metals like Ni in the multimetal-contaminated substrate that is usually found at anthropogenically contaminated, metalliferous sites. In our experiments, we were able to extract, choosing the right conditions, approximately 0.5 g Co or Cd per hectare and 4.5 and 1.2 g Ni and Sr per hectare, respectively, and at the same time, 15 mg of highly detrimental U and 120 g Mn per hectare. This multielement remediation can provide a suitable method to stabilize contaminated land and provide future land use, potentially with alternating extraction and renewable energy plant production cycles.

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Conflict of interest There are no potential conflicts of interest.

Compliance with ethical standards This research does not involve human participants or animals.

References

Abbas A, Edwards C (1989) Effects of metals on a range of *Streptomyces* species. Appl Env Microbiol 55:2030–2035

Abdel-Fattah GM, Mohamedin AH (2000) Interactions between a vesicular-arbuscular mycorrhizal fungus (*Glomus intraradices*) and *Streptomyces coelicolor* and their effects on sorghum plants grown in soil amended with chitin of brawn scales. Biol Fertil Soils 32:401–409

Abou-Shanab RA, Ghanem K, Ghanem N, Al-Kolaibe A (2008) The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. World J Microbiol Biotechnol 24:253–262

Adriano-Anaya ML, Salvador-Figueroa M, Ocampo JA, Garcia-Romera I (2006) Hydrolytic enzyme activities in maize (*Zea mays*) and sorghum (*Sorghum bicolor*) roots inoculated with *Gluconacetobacter diazotrophicus* and *Glomus intraradices*. Soil Biol Biochem 38: 879–886

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals—concepts and applications. Chemosphere 91:869–881

Ames RN, Reid CPP, Ingham ER (1984) Rhizosphere bacterial population responses to root colonization by a vesicular arbuscular mycorrhizal fungus. New Phytol 96:555–563

Amoroso MJ, Schubert D, Mitscherlich P, Schumann P, Kothe E (2000) Evidence for high affinity nickel transporter genes in heavy metal resistant *Streptomyces spec*. J Basic Microbiol 40:295–301

Arthur EL, Rice PJ, Rice PJ, Anderson TA, Baladi SM, Henderson KLD, Coats JR (2005) Phytoremediation—an overview. Crit Rev Plant Sci 24:109–122

Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of *in situ* heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Res Conserv Recycl 11:41–49

Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB, Scheckel K (2014) Remediation of heavy metal(-loid)s contaminated soils—to mobilize or to immobilize? J Hazard Mat 266:141–166

Brunetti G, Farrag K, Rovira PS, Nigro F, Senesi N (2011) Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by *Brassica napus* from contaminated soils in the Apulia region, Southern Italy. Geoderma 160:517–523

Büchel G, Bergmann H, Ebenå G, Kothe E (2005) Geomicrobiology in remediation of mine waste. Chem Erde – Geochem 65:1–5

Ciura J, Poniedzialek M, Sekara A, Jedrszczyk E (2005) The possibility of using crops as metal phytoremediants. Pol J Env Stud 14:17–22

Dimkpa CO, Svatos A, Dabrowska P, Schmidt A, Boland W, Kothe E (2008) Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in *Streptomyces* spp. Chemosphere 74:19–25

Dimkpa CO, Merten D, Svatos A, Buchel G, Kothe E (2009a) Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. J Appl Microbiol 107:1687–1696

Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009b) Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. Soil Biol Biochem 41: 154–162

Dushenkov S, Kapulnik Y, Blaylock M, Sorochisky B, Raskin I, Ensley B (1997) Phytoremediation: a novel approach to an old problem. In: Wise DL (ed) Studies in environmental science. Elsevier, Amsterdam, pp 563–572

Epelde L, Mijangos I, Becerril JM, Garbisu C (2009) Soil microbial community as bioindicator of the recovery of soil functioning derived from metal phytoextraction with sorghum. Soil Biol Biochem 41:1788–1794

Ernst WHO (2005) Phytoextraction of mine wastes—options and impossibilities. Chem Erde – Geochem 65:29–42

Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 86: 528–534

Grawunder A, Lonschinski M, Merten D, Büchel G (2009) Distribution and bonding of residual contamination in glacial sediments at the former uranium mining leaching heap of Gessen/Thuringia, Germany. Chem Erde – Geochem 69S2:5–19

Griffioen WAJ (1994) Characterization of a heavy metal-tolerant endomycorrhizal fungus from the surroundings of a zinc refinery. Mycorrhiza 4:197–200

Haferburg G, Kothe E (2007) Microbes and metals: interactions in the environment. J Basic Microbiol 47:453–467

Hernández-Allica J, Becerril JM, Garbisu C (2008) Assessment of the phytoextraction potential of high biomass crop plants. Env Poll 152: 32–40

Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207

Langella F, Grawunder A, Stark R, Weist A, Merten D, Haferburg G, Büchel G, Kothe E (2014) Microbially assisted phytoremediation approaches for two multi-element contaminated sites. Env Sci Poll Res 21:6845–6858

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Env Qual 31:109–120

Marchiol L, Fellet G, Perosa D, Zerbi G (2007) Removal of trace metals by *Sorghum bicolor* and *Helianthus annuus* in a site polluted by industrial wastes: a field experience. Plant Physiol Biochem 45: 379–387

Marques APGC, Moreira H, Franco AR, Rangel AOSS, Castro PML (2013) Inoculating *Helianthus annuus* (sunflower) grown in zinc and cadmium contaminated soils with plant growth promoting bacteria—effects on phytoremediation strategies. Chemosphere 92:74–83

McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. Curr Opin Biotechnol 14:277–282

McGrath SP, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232:207–214

Murillo JM, Maranon T, Cabrera F, Lopez R (1999) Accumulation of heavy metals in sunflower and sorghum plants affected by the Guadiamar spill. Sci Total Env 242:281–292

Neagoe A, Ebena G, Carlsson E (2005) The effect of soil amendments on plant performance in an area affected by acid mine drainage. Chem Erde – Geochem 65:115–129

Neagoe A, Stancu P, Onete M, Bodescu F, Gheorghe R, Iordache V (2014) Effects of arbuscular mycorrhizal fungi on *Agrostis capillaris* grown on amended mine tailing substrate at pot, lysimeter, and field plot scales. Environ Sci Pollut Res 21:6859–6876

Nogueira MA, Nehls U, Hampp R, Poralla K, Cardoso E (2007) Mycorrhiza and soil bacteria influence extractable iron and manganese in soil and uptake by soybean. Plant Soil 298:273–284

Pilon-Smits E (2005) Phytoremediation. Ann Rev Plant Biol 56:15–39

Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226

Rojas-Tapias D, Bonilla R, Dussán J (2012) Effect of inoculation with plant growth-promoting bacteria on growth and copper uptake by sunflowers. Water Air Soil Pollut 223:643–654

Saraswat S, Rai JPN (2009) Phytoextraction potential of six plant species grown in multimetal contaminated soil. Chem Ecol 25:1–11

Schindler F, Gube M, Kothe E (2012) Bioremediation and heavy metal uptake: microbial applications at field scale. In: Kothe E, Varma A (eds) Bio-geo interactions in metal contaminated soils. Springer, Heidelberg, pp 365–384

Schmidt A, Haferburg G, Sineriz M, Merten D, Büchel G, Kothe E (2005) Heavy metal resistance mechanisms in actinobacteria for survival in AMD contaminated soils. Chem Erde – Geochem 65: 131–144

Schmidt A, Haferburg G, Schmidt A, Lischke U, Merten D, Ghergel F, Büchel G, Kothe E (2009) Heavy metal resistance to the extreme: *Streptomyces* strains from a former uranium mining area. Chem Erde – Geochem 69:35–44

Schreiner RP, Koide RT (1993) Streptomycin reduces plant response to mycorrhizal infection. Soil Biol Biochem 25:1131–1133

Schütze E, Klose M, Merten D, Nietzsche S, Senftleben D, Roth M, Kothe E (2014) Growth of streptomycetes in soil and their impact on bioremediation. J Hazard Mater 267:128–135

Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K, Puschenreiter M (2013) The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol Biochem 60:182–194

Sheng X, Sun L, Huang Z, He L, Zhang W, Chen Z (2012) Promotion of growth and Cu accumulation of bio-energy crop (*Zea mays*) by bacteria: implications for energy plant biomass production and phytoremediation. J Env Manag 103:58–64

Sullivan TS, McBride MB, Thies JE (2013) Rhizosphere microbial community and Zn uptake by willow (*Salix purpurea* L.) depend on soil sulfur concentrations in metalliferous peat soils. Appl Soil Ecol 67: 53–60

Toler HD, Morton JB, Cumming JR (2005) Growth and metal accumulation of mycorrhizal sorghum exposed to elevated copper and zinc. Water Air Soil Poll 164:155–172

Turnau K, Ryszka P, Gianinazzi-Pearson V, van Tuinen D (2001) Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. Mycorrhiza 10: 169–174

Usman ARA, Mohamed HM (2009) Effect of microbial inoculation and EDTA on the uptake and translocation of heavy metal by corn and sunflower. Chemosphere 76:893–899

Vamerali T, Bandiera M, Mosca G (2009) Field crops for phytoremediation of metal-contaminated land. Env Chem Lett 8: 1–17

Violante A, Cozzolino V, Perelomov L, Caporale AG, Pigna M (2010) Mobility and bioavailability of heavy metals and metalloids in soil environments. J Soil Sci Plant Nutr 10:268–292

Wang FY, Lin XG, Yin R (2007) Inoculation with arbuscular mycorrhizal fungus *Acaulospora mellea* decreases Cu phytoextraction by maize from Cu-contaminated soil. Pedobiologia 51:99–109

Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J (2009) Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends Biotechnol 27:591–598

Yang X, Feng Y, He Z, Stoffella PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353

Yoon J, Cao X, Zhou Q, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Env 368:456–464

Zeien H, Brümmer G (1989) Chemische Extraktionen zur Bestimmung von Schwermetallbindungsformen in Böden. Mitt Dtsch Bodenkundl Ges 59:505–510

Zhao FJ, Lombi E, McGrath SP (2003) Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant Soil 249:37–43

Zhuang P, Yang QW, Wang HB, Shu WS (2007a) Phytoextraction of heavy metals by eight plant species in the field. Water Air Soil Poll 184:235–242

Zhuang X, Chen J, Shim H, Bai Z (2007b) New advances in plant growth-promoting rhizobacteria for bioremediation. Env Int 33: 406–413

Zhuang P, Shu W, Li Z, Liao B, Li J, Shao J (2009) Removal of metals by sorghum plants from contaminated land. J Env Sci 21:1432–1437

3.3 Manuscript III

Impact of soil microbes on remediation potential of high biomass crop plants

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Impact of soil microbes on remediation potential of high biomass crop plants

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Phytoremediation, Heavy metals, Pot experiments, Mycorrhiza*, Streptomyces*

Abstract

Pollution with heavy metals is one of the most severe environmental problems in soils affected by mining and industrial activities. The remediation using green plants as metal extractors or stabilizers provides an environmental friendly and cost-effective technology for metal contaminated sites. The impact of highly resistant *Streptomyces* strains and the mycorrhizal fungus *Rhizophagus irregularis* on heavy metal mobilization/stabilization and phytoremediation potential of the grasses *Andropogon gerardii* and *Sorghum bicolor*, and

the herbaceous perennial *Silphium perfoliatum* grown in multi-metal contaminated soil was evaluated. Biomass production and metal uptake into plant shoots were influenced by the applied soil microorganisms. The bioavailability of most metals was considerably decreased by addition of both bacterial amendments and mycorrhiza, but also found to be plant species and element specific. Thus, these findings indicated the potential of microbial application in enhanced phytoremediation of multi-element contaminated soils by high biomass plant species.

1. Introduction

The accumulation of heavy metals in soils of mining sites or agricultural land constitutes a potential risk for environmental and human health [1]. Elevated metal concentrations affect important soil functions including soil structure and its biological activity, and tend to inhibit growth of native plants [2]. Established remediation methods (e.g. excavation of contaminated soil material) are mostly expensive, site disruptive and only appropriate for decontamination of small soil volumes [3]. Plant-based soil remediation, known as phytoremediation, is a low-cost and environmentally friendly alternative for removing heavy metals through uptake and accumulation into harvestable biomass (phytoextraction) or reducing mobility, toxicity and dispersion of metals (phytostabilization) in contaminated areas [for review see: 4]. Depending on remediation strategy, applied plants should either exhibit the ability to translocate high amounts of toxic metals to harvestable plant tissues in combination with a high biomass production [5, 6], or to immobilize heavy metals within the root zone through adsorption or accumulation in an extensive, well-branched root system [7]. Both strategies offer a passive *in situ* technique with reduced environmental impact for partial soil decontamination, site restoration, reduction of leaching and metal mobility, and may even allow for harvesting of economically valuable plant biomass. For a successful soil remediation, two major

components are of high importance: soil conditions prevalent at the site including soil metal bioavailability, and choice of plants and their symbiotic rhizosphere microbes. The use of soil microorganisms with phytoremediation approaches might improve plant performance, metal tolerance and accumulation [8, 9], but also may exert an impact on soil conditions [10]. Rhizosphere microorganisms are known to play a crucial role in contaminant availability by altering soil properties (e.g., pH and Eh), reduction of metal toxicity, and, most importantly, metal accumulation in plant biomass [5]. They positively affect plant health and growth by providing nutrients, including nitrogen, phosphorus and iron [11], release of phytohormones [12], production of iron chelating compounds and siderophores [13], synthesis of ACC deaminase [14] or by improving the plant's ability to resist pathogen attack [15].

The physiological properties of plant-associated rhizobacteria not only allow for improved plant growth on metalliferrous soils, they also contribute to soil metal bioavailability and, thus, play a significant role in phytoremediation processes [16]. Heavy metal resistant soil bacteria, specifically Gram-positive streptomycetes, have been successfully applied in bioremediation studies [17]. For instance, *Streptomyces acidiscabies* has been found to enhance growth of cowpea plants by siderophore production [18], while *Streptomyces mirabilis* could stimulate biomass productivity of *Sorghum bicolor* in heavy metal contaminated soil [19].

The use of fast-growing, high biomass crop plants for phytoremediation purposes offer several advantages like high resistance to adverse conditions and metal accumulation potential [for review: 20]. In this work, the role of metal-resistant *Streptomyces* strains and the arbuscular mycorrhizal fungus *Rhizophagus irregularis* on plant performance and metal mobility in contaminated substrate were investigated. The aim of the current study was to compare the remediation potential of the grasses *Andropogon gerardii* and *Sorghum bicolor* with the high biomass crop plant *Silphium perfoliatum* growing in a multi-

metal contaminated substrate, and to evaluate the impact of microbial amendment in pot experiments.

2. Material and Methods

2.1 Soil sampling and analyses

Heavy metal contaminated soil from 5 to 10 cm depth was collected from the test site Gessenwiese in the former uranium mining district Ronneburg in Eastern Thuringia, Germany [21]. The potting substrate was air-dried and sieved to a maximum grain size of 2 mm for soil analysis. Soil pH(H_2O) was determined after shaking a 1:4 suspension for 1 h, left to settle for 24 h and measured using pH330 (WTW). The same solution was used to determine electrical conductivity (EC; TetraCon 325 and LF320, WTW). Total amounts of heavy metals were determined by digesting 100 mg of soil (40 % HF, 70 % $HClO_4$, and 65 % HNO_3) in a pressure digestion system (DAS 30, PicoTrace). Bioavailability of soil elements was determined following sequential extraction [22]. 2 g of air-dried soil material was mixed with 1 M NH_4NO_3 solution (p.a., Merck) to extract the mobile fraction (F1) of soil metals. Element contents were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES; 725 ES, Varian) and inductively coupled plasma-mass spectrometry (ICP-MS, X-Series II, Thermo Fisher Scientific) in triplicates. Total contents and metal concentrations for mobile fraction (F1) of examined soil are added as values before planting (t_0 at day 0), where t_0 was compared to soil metal composition after plant growth.

2.2 Microbial growth and metal resistance

Four heavy metal-resistant *Streptomyces* strains isolated from the former uranium mining area near Ronneburg, Germany [23, 24], were applied as bacterial inoculum in pot experiments. Identification of strain P18A-1 was based on 16S rDNA amplified using primer pair fD1 Forward (5'-AGAGTTTGATCCTGGCTCAG-3') and rP2 Reverse (5'-

107 ACGGCTACCTTGTTACGACTT-3') and sequenced (GATC Biotech, Konstanz,
108 Germany). *S. naganishii* P9A-1, *S. chromofuscus* P10A-4, *S. mirabilis* P16B-1 and
109 *Streptomyces sp.* P18A-1 were cultured on starch casein agar plates (10 g/l starch, 1 g/l
110 casein, 0.5 g/l K_2HPO_4, 15 g/l Agar, pH 7.0 – 7.5) for spore production. Spores were
111 collected after 7 days of incubation at 28 °C and quantified using a Thoma counting
112 chamber. Each strain was diluted in distilled water to prepare a solution containing
113 10^6 spores/ml. Heavy metal resistance was tested on minimal medium agar plates [25]
114 supplemented with single metal solutions of Al, Cd, Cu, Ni and Zn in rising concentrations.
115 Bacterial growth was evaluated after 10 days of cultivation at 28 °C. The arbuscular
116 mycorrhizal inoculum was obtained as expanded clay containing spores of *Rhizophagus*
117 *irregularis* (Biofa AG, Münsingen, Germany) with 100 spores per gram.
118
119 *2.3 Greenhouse experiments*
120 Pot experiments were carried out in the summer of 2014 in the greenhouse of the
121 Thüringer Landesanstalt für Landwirtschaft (TLL), Jena. The tested plants included two
122 grass species (*Andropogon gerardii* and *Sorghum bicolor*) and a herbaceous high yielding
123 crop plant (*Silphium perfoliatum*). Pots (12 x 12 x 16 cm) were filled with 2.5 kg of heavy
124 metal contaminated soil mixed with 250 ml deionized water. The grasses *A. gerardii* (12
125 seeds per pot; Jelitto Staudensamen, Germany), *S. bicolor* (10 seeds; KWS SAAT,
126 Germany) and the herb *S. perfoliatum* (4 seeds; N.L. Chrestensen, Germany) were tested
127 in four experimental treatments: unamended control (C), amended with *Streptomyces*
128 strains (S), amended with arbuscular mycorrhizal fungus (M) and amended with a mixture
129 of streptomycetes and the mycorrhizal fungus (MS). Each treatment was replicated three
130 times. Plants were inoculated with 5 ml of bacterial spore suspension containing 10^6
131 cfu/pot and/or 4 g of *R. irregularis* granulate at time of seeding. Uninoculated control
132 plants received 5 ml sterilized distilled water. The pots were covered with plastic wrap until
133 germination occurred. Plants grew with natural day/night rhythm at ambient temperature in

a range of 15 °C to 30 °C. The pots were arranged in a randomized pattern and randomly re-arranged every 4 days. All plants were watered daily with distilled water and above-ground biomass was harvested after a period of 12 weeks.

2.4 Plant harvest and analyses

After harvesting, plant shoots were washed three times with deionized water, and oven-dried at 30 °C until constant weight to determine shoots dry weight. Plants were then ground using an ultra-centrifugal mill (ZM100, Retsch) for 1 min to a size below 0.5 mm. For element analysis, up to 200 mg of plant material was weighted and digested with 5 ml HNO_3 (65 %, supra, Merck) in a microwave-pressure system (Mars 5 XPRESS, CEM Germany), heated for 15 min at 180 °C and then cooled for at least 90 min. The digested samples were transferred into 25 ml flasks filled up with ultra pure water (PureLab Plus, USF Elga) and analyzed for heavy metals by ICP-OES (725 ES, Varian) and ICP-MS (X-Series II, Thermo Fisher Scientific) in triplicates. The precision and accuracy of the ICP-MS and ICP-OES measurements were proven by analyzing standard reference material SPS-SW2 (Spectrapure Standards AS) and NIST 1643e (NIST) and by measuring multi element standard solution (500 mg/l Ca, K, Mg Bernd Kraft) each in dilution 1:5 (v:v) and comparison to the certified values. Typical precision for triplicate measurements were ≤ 2 % for ICP-MS and ≤ 5 % for ICP-OES.

2.5 Phytoremediation efficiency

Two indices were calculated to evaluate plants for their phytoremediation potential. The bioconcentration factor (BCF) for metal uptake was calculated by dividing concentration in harvested shoots by soil content in mobile fraction F1 and the transfer factor (TF) by dividing shoot metal concentration by total soil content.

159 *2.6 Statistical analyses*

160 Statistical analyses were performed with R 3.0.3 (R Development Core Team 2009,
161 http.//www.R-project.org). The data were analyzed for variance (ANOVA) with a
162 confidence level of 95 %. Significant differences between treatment means were
163 confirmed by Tukey's test ($P < 0.05$). Means and standard deviations were calculated
164 using Microsoft Excel 2007 (Microsoft Corporation).

165 **3. Results**

166 *3.1 Heavy metal resistance*

167 The four strains P9A-1, P10A-4, P16B-1 and P18A-1, all belonging to the genus
168 *Streptomyces*, were tested for their resistance to heavy metals supplied in minimal
169 medium (Fig. 1). All strains showed high resistance to Ni and Zn, while Al and Cu were
170 tolerated only at low concentrations. *S. mirabilis* P16B-1 was able to grow on high
171 concentrations of Ni up to 35 mM. In the presence of Al, *S. chromofuscus* P10A-4
172 tolerated concentrations of 12 mM. The tested *Streptomyces* strains showed no growth in
173 the presence of Cd at concentrations higher than 0.5 mM, except *Streptomyces sp.* P18A-
174 1; this strain also showed the ability to cope with 110 mM Zn.

175

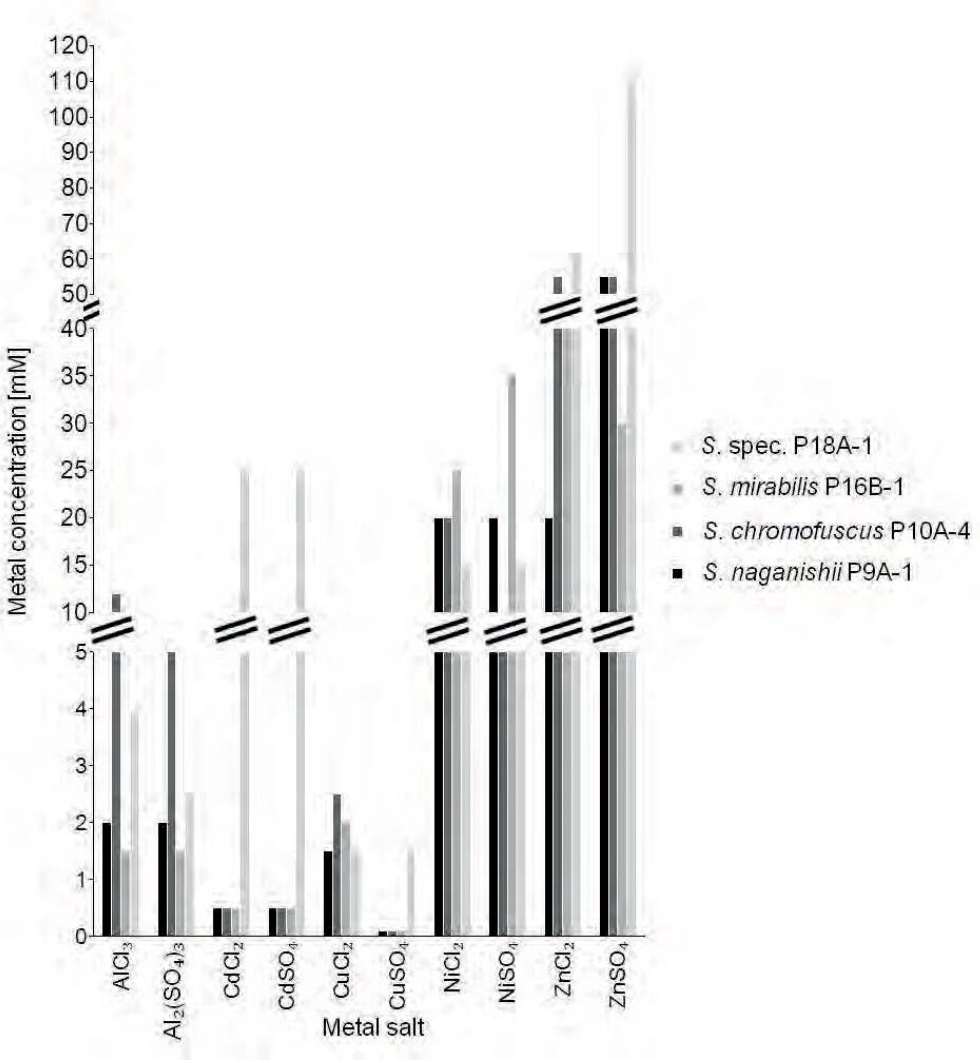

Fig. 1: Heavy metal resistance of *Streptomyces* strains on minimal medium [mM metal salt]

3.2 Plant biomass production

The influence of microbial inoculation on *S. bicolor*, *S. perfoliatum* and *A. gerardii* growth was evaluated after 12 weeks by measuring above-ground biomass (Fig. 2). *S. bicolor* showed the highest biomass production, significantly larger than for *S. perfoliatum* and *A. gerardii*. The lowest biomass productivity was recorded for the grass *A. gerardii*. Shoot biomass of inoculated plants that were treated with *Streptomyces* strains was significantly increased after 12 weeks of plant growth.

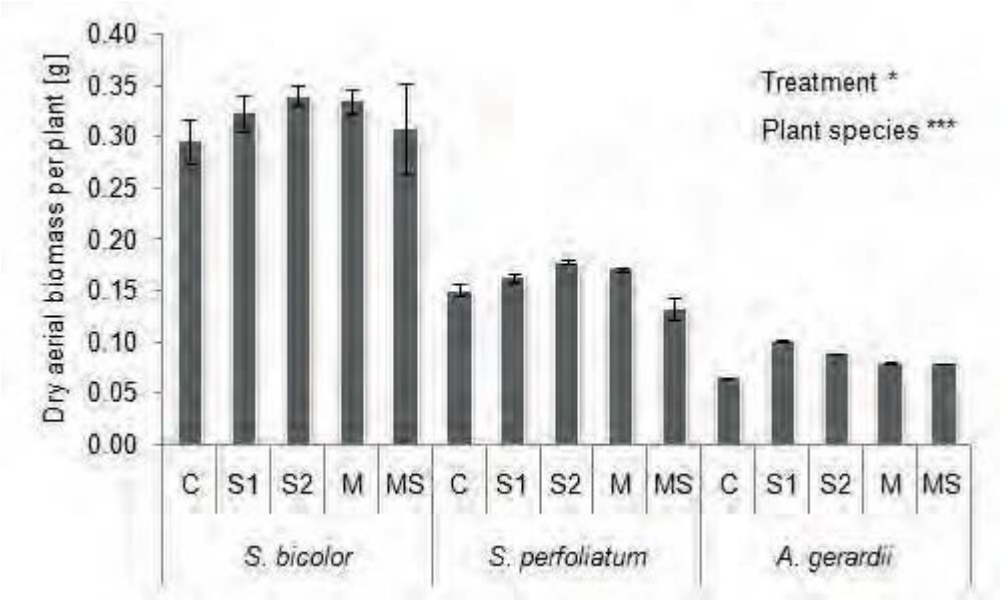

Fig. 2: Treatment effects on above-ground biomass of plants grown in metal-contaminated potting substrate. Shoot dry weight per plant (means ± SD) is given for *S. bicolor* (n = 10), *S. perfoliatum* (n = 4) and *A. gerardii* (n = 12) after 12 weeks. Two-way ANOVA was performed to determine the influence of microbial treatment and plant species. Significance levels: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. C, control treatment without inoculation; S, amended with streptomycetes; M, amended with mycorrhiza; MS, amended with mycorrhiza and streptomycetes.

3.3 Metal accumulation in plant shoots

In general, metal uptake into shoot biomass differed significantly between tested plant species (Tab. 1). *S. perfoliatum* and the grass *A. gerardii* accumulated significantly more Al, Mn, Ni, Sr and Zn than *S. bicolor*. In case of Cd, high amounts were found in non-treated shoots of *S. perfoliatum* and *A. gerardii*, while microbial inoculation increased Cd uptake in *S. bicolor*. Shoot concentrations of Co and Cu were significantly decreased in inoculated treatments. Mn was most effectively accumulated in shoots of *S. perfoliatum*,

205 but no contribution of microbial amendments could be observed. *S. perfoliatum* also
206 extracted more than threefold the amount of Ni than the grasses. In contrast, application
207 of either mycorrhiza or a mixture of mycorrhiza and streptomycetes decreased the shoot
208 metal uptake by *S. perfoliatum* and *A. gerardii*, particularly for Ni and Sr. However, this
209 effect was not the same for *S. bicolor*. Low levels of U were found in shoots of all plants.
210
211 Tab. 1: Shoot metal concentrations (mg kg^{-1}) in harvestable plant biomass

	Treatments	Al	Cd	Co	Cu	Mn	Ni	Sr	U	Zn
S. bicolor	Control	26.8 ± 2.2	0.84 ± 0.02	0.43 ± 0.02	4.1 ± 0.3	256 ± 21	16.2 ± 1.3	4.9 ± 0.1	0.01 ± 0.00	16.0 ± 1.4
	Streptomyces P9A-1	25.1 ± 5.1	0.89 ± 0.09	0.52 ± 0.12	4.0 ± 0.2	263 ± 5	20.0 ± 5.7	4.8 ± 0.3	0.01 ± 0.00	17.1 ± 4.8
	Streptomyces P10A-4	23.3 ± 1.8	0.90 ± 0.16	0.40 ± 0.05	3.8 ± 0.1	282 ± 23	12.9 ± 0.7	4.9 ± 0.1	0.01 ± 0.00	18.8 ± 0.9
	Mycorrhiza	23.6 ± 2.9	1.09 ± 0.10	0.40 ± 0.02	4.0 ± 0.2	269 ± 11	14.6 ± 1.0	4.8 ± 0.4	0.01 ± 0.00	18.8 ± 1.3
	Mycorrhiza + *Streptomyces*	23.3 ± 3.2	1.09 ± 0.09	0.39 ± 0.00	3.9 ± 0.1	278 ± 22	12.9 ± 0.9	4.7 ± 0.1	0.01 ± 0.00	16.5 ± 3.1
S. perfoliatum	Control	804 ± 339	2.9 ± 0.3	3.1 ± 0.6	11.2 ± 0.9	1236 ± 128	163 ± 10	23.9 ± 2.2	0.45 ± 0.20	99 ± 12
	Streptomyces P18A-1	397 ± 133	2.5 ± 0.3	1.7 ± 0.4	8.2 ± 0.4	1241 ± 68	136 ± 22	21.2 ± 1.5	0.15 ± 0.05	86 ± 4
	Streptomyces P16B-1	445 ± 89	2.3 ± 0.9	1.7 ± 0.2	8.4 ± 0.6	972 ± 88	119 ± 6	17.6 ± 0.7	0.34 ± 0.17	78 ± 16
	Mycorrhiza	426 ± 120	2.3 ± 0.2	1.7 ± 0.1	9.7 ± 1.7	1081 ± 62	125 ± 11	18.1 ± 2.4	0.18 ± 0.05	72 ± 11
	Mycorrhiza + *Streptomyces*	438 ± 220	2.1 ± 0.4	2.1 ± 0.5	8.8 ± 0.5	992 ± 80	134 ± 16	16.5 ± 1.4	0.22 ± 0.12	61 ± 16
A. gerardii	Control	404 ± 136	0.95 ± 0.27	0.93 ± 0.20	4.1 ± 0.9	519 ± 31	42.6 ± 6.7	8.4 ± 0.7	0.37 ± 0.13	47.7 ± 16.0
	Streptomyces P18A-1	763 ± 211	0.93 ± 0.10	1.34 ± 0.33	4.7 ± 0.6	548 ± 43	47.5 ± 3.3	9.1 ± 0.2	0.60 ± 0.13	45.2 ± 5.3
	Streptomyces P16B-1	221 ± 9	0.61 ± 0.13	0.57 ± 0.01	2.9 ± 0.2	442 ± 7	30.9 ± 0.8	7.6 ± 0.4	0.13 ± 0.01	33.5 ± 4.7
	Mycorrhiza	301 ± 118	0.81 ± 0.17	0.75 ± 0.24	3.5 ± 0.6	548 ± 97	38.8 ± 7.4	7.6 ± 0.3	0.29 ± 0.20	44.1 ± 15.7

	Mycorrhiza + *Streptomyces*	255 ± 33	0.83 ± 0.14	0.73 ± 0.08	3.4 ± 0.4	530 ± 51	31.6 ± 1.3	6.7 ± 0.3	0.20 ± 0.01	39.6 ± 5.3
Significance	Treatment	n.s.	n.s.	**	**	n.s.	***	***	n.s.	n.s.
	Plant	***	***	***	***	***	***	***	***	***
	Treatment x Plant	n.s.	n.s.	*	*	n.s.	n.s.	*	*	n.s.

* P < 0.05; ** P < 0.01; *** P < 0.001; n.s. non-significant at the level P < 0.05.

3.4 Factors for transfer (TF) and bioconcentration (BCF) of heavy metals

In order to evaluate the remediation potential of the tested plant species, the transfer and bioconcentration factors were calculated. All plants showed transfer factors > 1 for Cd into shoot biomass, with highest extraction into *S. perfoliatum* also extracting Mn, Ni and Zn (Tab. 2). Microbial inoculation enhanced Cd uptake into *S. bicolor*, while transfer of Co, Cu, Ni and Sr into plant shoots was significantly reduced by applying microbial amendments. Exclusion was found for Al, Co, Sr and U. Since bioavailable metal contents in examined soil substrate are extremely high, bioaccumulation of toxic elements could be observed. *S. perfoliatum* showed the highest extraction capacity for all elements (Tab. 3). However, no significant contribution of microbial inoculation could be found. The presence of bacterial amendments increased uptake of Al, Co and U into *A. gerardii*, while metal extraction was mainly reduced into *S. perfoliatum*. Conversely, a significant extraction of Co and Ni occurred into *S. bicolor* amended with microbial inoculants.

Tab. 2: Transfer of soil metals to plant shoots (shoot metal concentration/total soil content)

	Treatments	Al	Cd	Co	Cu	Mn	Ni	Sr	U	Zn
S. bicolor	Control	< 0.01	1.31	0.02	0.14	0.41	0.32	0.05	< 0.01	0.23
	Streptomyces P9A-1	< 0.01	1.39	0.03	0.14	0.42	0.39	0.05	< 0.01	0.24
	Streptomyces P10A-4	< 0.01	1.41	0.02	0.13	0.45	0.25	0.05	< 0.01	0.27
	Mycorrhiza	< 0.01	1.69	0.02	0.14	0.43	0.29	0.05	< 0.01	0.27
	Mycorrhiza + *Streptomyces*	< 0.01	1.71	0.02	0.13	0.44	0.25	0.04	< 0.01	0.24
S.	Control	0.01	4.58	0.18	0.38	1.96	3.22	0.23	0.09	1.42

perfoliatum	*Streptomyces* P18A-1	0.01	3.86	0.10	0.28	1.97	2.68	0.20	0.03	1.22
	Streptomyces P16B-1	0.01	3.65	0.09	0.29	1.54	2.34	0.17	0.07	1.11
	Mycorrhiza	0.01	3.58	0.10	0.33	1.71	2.46	0.17	0.04	1.02
	Mycorrhiza + *Streptomyces*	0.01	3.27	0.12	0.30	1.57	2.65	0.16	0.04	0.87
A. gerardii	Control	0.01	1.48	0.05	0.14	0.82	0.84	0.08	0.07	0.68
	Streptomyces P18A-1	0.01	1.45	0.08	0.16	0.87	0.94	0.09	0.12	0.65
	Streptomyces P16B-1	0.00	0.95	0.03	0.10	0.70	0.61	0.07	0.03	0.48
	Mycorrhiza	0.01	1.27	0.04	0.12	0.87	0.77	0.07	0.06	0.63
	Mycorrhiza + *Streptomyces*	0.00	1.29	0.04	0.12	0.84	0.62	0.06	0.04	0.57
Significance	Treatment	n.s.	n.s.	**	**	n.s.	***	***	n.s.	n.s.

230 * P < 0.05; ** P < 0.01; *** P < 0.001; n.s. non-significant at the level P < 0.05.

231

232 Tab. 3: Bioconcentration factors of heavy metals (shoot metal concentration/soil content in
233 mobile fraction F1)

	Treatments	Al	Cd	Co	Cu	Mn	Ni	Sr	U	Zn
S. bicolor	Control	0.58	5.50	0.82	10.68	3.51	1.90	1.44	0.31	5.45
	Streptomyces P9A-1	0.55	5.83	1.00	10.42	3.61	2.35	1.43	0.29	5.80
	Streptomyces P10A-4	0.51	5.93	0.78	9.85	3.87	1.51	1.46	0.22	6.39
	Mycorrhiza	0.51	7.13	0.78	10.47	3.69	1.71	1.41	0.22	6.38
	Mycorrhiza + *Streptomyces*	0.51	7.17	0.75	10.11	3.81	1.51	1.39	0.22	5.63
S. perfoliatum	Control	17.51	19.25	6.08	29.28	16.95	19.18	7.05	14.38	33.82
	Streptomyces P18A-1	8.65	16.23	3.32	21.32	17.03	15.92	6.27	4.83	29.09
	Streptomyces P16B-1	9.69	15.36	3.21	21.91	13.33	13.95	5.19	10.64	26.41
	Mycorrhiza	9.29	15.05	3.23	25.15	14.83	14.65	5.35	5.74	24.33
	Mycorrhiza + *Streptomyces*	9.54	13.76	4.01	22.88	13.61	15.74	4.88	6.84	20.76
A. gerardii	Control	8.80	6.20	1.81	10.62	7.11	4.99	2.47	11.69	16.21
	Streptomyces P18A-1	16.61	6.09	2.60	12.32	7.52	5.57	2.70	18.94	15.37
	Streptomyces P16B-1	4.81	4.00	1.11	7.56	6.06	3.62	2.25	4.16	11.38
	Mycorrhiza	6.56	5.34	1.46	9.23	7.52	4.55	2.25	9.28	15.01
	Mycorrhiza + *Streptomyces*	5.55	5.42	1.41	8.90	7.28	3.71	1.98	6.25	13.46
Significance	Treatment	n.s.	n.s.	**	**	n.s.	***	***	n.s.	n.s.

234 * P < 0.05; ** P < 0.01; *** P < 0.001; n.s. non-significant at the level P < 0.05.

235

236 *3.5 Impact on metal loads and availability*

237 Heavy metal availability at contaminated sites is strongly influenced by pH, but also plant
238 growth and soil microorganisms can affect metal solubility and mobilization. To test the
239 effects of planting and microbial inoculation on alteration of metal contents in the
240 respective substrate, changes in bioavailable metal loads were compared (Tab. 4). The
241 substrate showed multi-metal contamination with relatively high bioavailable fractions of
242 Cd, Mn and Ni at pH 4.3 to 4.7 and an electrical conductivity of 471 ± 117 µS cm^{-1}. Most
243 metals were remarkably reduced at the end of the growing season, especially in pots
244 planted with *S. bicolor*. Additionally, a decrease in bioavailable fractions of Al and Cu was
245 induced by bacterial and mycorrhizal inoculation. A significant reduction of Co availability
246 occurred into *S. perfoliatum* pots amended with a mixture of mycorrhiza and
247 streptomycetes. Microbial inoculation significantly reduced Sr bioavailability in case of *A.*
248 *gerardii*. A slight reduction of the mobile fraction of Zn could be found in amended pots for
249 all plant species. In contrast, a mobilization of Al and Cu occurred for inoculated *Silphium*,
250 while untreated *A. gerardii* mobilized Ni from the mobile fraction. Both plant species also
251 showed a low mobilization of U during the experiment.

252

253 Tab. 4: Alteration of soil metal bioavailability after plant growth (mg kg^{-1} dry soil)

	Treatments	Al	Cd	Co	Cu	Mn	Ni	Sr	U	Zn
Before	Total soil content	54374 ± 3042	0.64 ± 0.18	17.5 ± 3.1	29.3 ± 1.4	631 ± 167	50.8 ± 3.5	106 ± 6	5.2 ± 1.4	69.9 ± 3.4
	Bioavailable	45.9 ± 15.2	0.15 ± 0.04	0.52 ± 0.24	0.38 ± 0.07	72.9 ± 10.8	8.5 ± 2.0	3.4 ± 0.3	0.03 ± 0.02	2.9 ± 0.6
S. bicolor	Control	41.6 ± 1.3	0.09 ± 0.00	0.29 ± 0.02	0.44 ± 0.10	39.4 ± 1.3	5.9 ± 0.3	3.4 ± 0.1	0.01 ± 0.00	2.2 ± 0.2
	Streptomyces P9A-1	39.3 ± 0.7	0.10 ± 0.01	0.39 ± 0.04	0.35 ± 0.01	46.9 ± 2.4	6.2 ± 0.3	3.5 ± 0.1	0.01 ± 0.00	2.1 ± 0.1
	Streptomyces P10A-4	39.6 ± 0.4	0.09 ± 0.01	0.29 ± 0.03	0.34 ± 0.04	41.3 ± 3.5	6.0 ± 0.1	3.5 ± 0.1	0.01 ± 0.00	2.1 ± 0.1
	Mycorrhiza	39.5 ± 1.8	0.09 ±	0.37 ±	0.31 ±	46.4 ± 3.2	5.9 ± 0.6	3.4 ±	0.01 ±	1.9 ± 0.2

			0.01	0.03	0.01			0.1	0.00	
	Mycorrhiza + *Streptomyces*	36.6 ± 3.7	0.09 ± 0.00	0.35 ± 0.03	0.32 ± 0.03	45.0 ± 2.4	6.1 ± 0.2	3.6 ± 0.1	0.01 ± 0.00	2.1 ± 0.1
S. perfoliatum	Control	70.1 ± 1.4	0.18 ± 0.01	0.21 ± 0.02	0.51 ± 0.04	47.1 ± 2.0	11.1 ± 0.5	2.9 ± 0.1	0.06 ± 0.00	2.5 ± 0.1
	Streptomyces P18A-1	74.9 ± 1.9	0.17 ± 0.01	0.20 ± 0.02	0.57 ± 0.01	42.2 ± 2.6	11.2 ± 0.4	2.9 ± 0.1	0.06 ± 0.00	2.9 ± 0.4
	Streptomyces P16B-1	73.7 ± 1.4	0.16 ± 0.02	0.18 ± 0.04	0.51 ± 0.01	41.1 ± 7.2	11.1 ± 0.4	2.8 ± 0.1	0.06 ± 0.00	2.5 ± 0.2
	Mycorrhiza	77.2 ± 0.9	0.17 ± 0.00	0.18 ± 0.04	0.52 ± 0.01	41.0 ± 2.6	11.2 ± 0.3	3.0 ± 0.1	0.06 ± 0.00	2.4 ± 0.0
	Mycorrhiza + *Streptomyces*	72.9 ± 1.4	0.16 ± 0.01	0.16 ± 0.03	0.48 ± 0.01	40.6 ± 3.0	10.7 ± 0.1	3.1 ± 0.3	0.06 ± 0.00	2.4 ± 0.0
A. gerardii	Control	69.6 ± 3.0	0.20 ± 0.01	0.25 ± 0.02	0.55 ± 0.05	55.1 ± 2.5	12.3 ± 0.5	3.0 ± 0.1	0.06 ± 0.00	2.9 ± 0.2
	Streptomyces P18A-1	66.0 ± 0.9	0.18 ± 0.01	0.23 ± 0.01	0.50 ± 0.01	50.1 ± 2.2	11.2 ± 0.1	2.9 ± 0.0	0.05 ± 0.00	2.6 ± 0.1
	Streptomyces P16B-1	64.1 ± 1.7	0.16 ± 0.01	0.22 ± 0.02	0.49 ± 0.00	48.0 ± 2.3	10.8 ± 0.1	2.8 ± 0.0	0.05 ± 0.00	2.9 ± 0.3
	Mycorrhiza	65.3 ± 2.7	0.17 ± 0.00	0.18 ± 0.01	0.44 ± 0.02	43.4 ± 1.7	10.5 ± 0.2	2.8 ± 0.2	0.05 ± 0.00	2.3 ± 0.2
	Mycorrhiza + *Streptomyces*	65.6 ± 2.4	0.20 ± 0.00	0.28 ± 0.03	0.48 ± 0.02	59.4 ± 5.5	11.9 ± 0.1	2.9 ± 0.0	0.06 ± 0.00	2.7 ± 0.1
Significance	Treatment	n.s.	n.s.	**	**	n.s.	***	***	n.s.	n.s.
	Plant	***	***	***	***	***	***	***	***	***
	Treatment x Plant	n.s.	n.s.	*	*	n.s.	n.s.	*	*	n.s.

* P < 0.05; ** P < 0.01; *** P < 0.001; n.s. non-significant at the level P < 0.05.

4. Discussion

Finding optimal plant species and selection of microbial amendments contributing to plant performance thus enhancing the total uptake of toxic soil metals are important requirements for a successful remediation of heavy metal polluted soils [26]. Fast-growing, high biomass crop species have been extensively investigated for phytoremediation purposes [27]. In our pot experiments, we evaluated the impact of

262 microbial amendments on metal removal by two grasses and one herbaceous crop
263 species grown on multi-metal contaminated substrate derived from a former uranium
264 mining site. Their efficiency of metal removal and biomass productivity was compared to
265 assess the possibility of using these plant species as phytoremediation plants. Metal
266 resistant streptomycetes, isolated from the same metal-contaminated site [24], and/or the
267 arbuscular mycorrhizal fungus *Rhizophagus irregularis* were applied to enhance plant
268 growth and to modify metal uptake.
269 The Gram-positive, aerobic soil bacteria have been shown to be able to combine different
270 mechanisms for heavy metal resistance for survival in highly contaminated soils [28]. All
271 *Streptomyces* strains showed the highest resistance against Ni and Zn; against cadmium,
272 only strain *Streptomyces sp.* P18A-1 was found to tolerate concentrations higher than 0.5
273 mM. It has been shown that the application of soil bacteria, specifically streptomycetes,
274 may positively affect plant growth on metalliferrous soils [19]. They contribute to plant
275 growth by release of phytohormones (indole acetic acid), excretion of siderophores or
276 other iron chelators with a high Fe^{3+} affinity, and solubilization of inorganic phosphates
277 [29]. Additionally, symbiosis with metal tolerant arbuscular mycorrhizal fungi, mostly of the
278 genus *Glomus*, has been found to affect plant tolerance to metals and biomass production
279 [30]. Mycorrhizal plants in metal-rich habitats profit from nutrient and water supplied
280 through the fungus, and protection of plant roots from heavy metals [5, 31]. The pot
281 experiments suggested that the average biomass production of the root grass *S. bicolor*
282 was clearly higher than that of *S. perfoliatum* and *A. gerardii*. The relatively low biomass
283 production of *Silphium* plants could be explained by the plant's typical growth dynamics,
284 involving rapidly increasing biomass yield only later in the years of growth [32].
285 An effect of microbial inoculation on biomass productivity was seen for all plant species.
286 This result is in line with earlier studies [33]. Inoculation with the mycorrhizal fungus
287 stimulated plant growth almost to the same extent as the bacterial amendments,
288 suggesting that mycorrhizal colonization of plant roots contributes to nutrient availability

289 and plant tolerance to heavy metals [34]. In contrast, antagonistic interactions between
290 arbuscular mycorrhizal fungi and actinomycetes have also been reported [35,36]. Since
291 the process of metal uptake and accumulation by different plant species strongly depends
292 on metal availability in soil, plant growth and tolerance [37], we assessed whether
293 inoculation with mycorrhizal fungus and streptomycetes affected metal uptake.
294 In our study, harvestable biomass of crop plants exclusively consisted of the aerial part,
295 since roots may not be considered for total plant extraction of metals from the soil. Metal
296 accumulation into shoot biomass of plants was affected by presence of the microbial
297 inoculum. The addition of streptomycetes significantly increased Co and Ni concentrations
298 in shoots of *S. bicolor*, while dual inoculation with bacteria and the mycorrhizal fungus had
299 no effect. This effect was seen in previous studies for *Sorghum* plants exposed to
300 elevated heavy metal concentrations [34]. In contrast, *S. perfoliatum* showed decreased
301 uptake of all elements with microbial application. In the literature, only few investigations
302 exist on metal tolerance and remediation potential of *S. perfoliatum* [38]. Bacterial
303 inoculation could partially enhance shoot metal concentration of *Andropogon* plants,
304 showing that inoculation with soil microbes could either increase or lower metal
305 concentrations in plant shoots.
306 Despite plants mechanisms, including phytosiderophores or root exudates [6] to stimulate
307 the solubility and availability of metals in the rhizosphere, the application of soil
308 microorganisms can strongly influence bioavailability of heavy metals to plants in soil [39].
309 The microbial inoculation had an effect on soil metal availability. A significant reduction of
310 the bioavailable soil fraction of Cu was found for *S. bicolor* and Co for *A.gerardii* in the
311 presence of mycorrhiza. The addition of a mixture of mycorrhiza and streptomycetes led
312 to a significant decrease of Co contents in the mobile soil fraction for *S. perfoliatum*, while
313 reduction of Sr bioavailability occurred for *Andropogon* inoculated with streptomycetes. On
314 the contrary, mobilization of metals, which is necessary for plant uptake, was seen with *S.*
315 *perfoliatum* and *A. gerardii* with respect to Al, Cu and Ni. These findings are in line with

316 other studies, reporting that inoculation with arbuscular mycorrhizal fungi or rhizospheric
317 bacteria could either enhance soil metal mobility [10, 40] or effectively immobilize metals
318 within the rhizosphere [34].
319 This study emphasizes the importance of soil microorganisms in phytoremediation of soil
320 contaminated by heavy metals. We could show that microbial inoculation can improve
321 plant performance and play a crucial role in altering soil metal availability for plant uptake.
322 Thus, our results confirmed the potential of microbial amendments in metal extraction as
323 well as stabilization by high biomass producing crop plants from metal-contaminated soil.
324 We thus provide a basis from which to assess the effects for new sites, where
325 performance always will be dependent upon both the plant species and the type of
326 microbial inoculum.
327

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333

334 **6. References**

335 [1] Garcia G, Penas JM, Manteca JI (2008): Zn mobility and geochemistry in surface
336 sulfide mining soils from SE Spain. Environmental Research 106, 333-339
337 [2] Pratas J, Prasad MNV, Freitas H, Conde L (2005): Plants growing in abandoned mines
338 of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten
339 and mine reclamation. Journal of Geochemical Exploration 85, 99-107
340 [3] Padmavathiamma PK, Li LY (2007): Phytoremediation Technology: Hyper-
341 accumulation metals in plants. Water, Air, and Soil Pollution 184, 105-126

342 [4] Arthur EL, Rice PJ, Rice PJ, Anderson TA, Baladi SM, Henderson KLD, Coats JR
343 (2005): Phytoremediation - an overview. Critical Reviews in Plant Sciences 24, 109-
344 122
345 [5] Ernst WHO (2005): Phytoextraction of mine wastes - Options and impossibilities.
346 Chemie Der Erde-Geochemistry 65, 29-42
347 [6] Saraswat S, Rai JPN (2009): Phytoextraction potential of six plant species grown in
348 multimetal contaminated soil. Chemistry and Ecology 25, 1-11
349 [7] Kucharski R, Sas-Nowosielska A, Malkowski E, Japenga J, Kuperberg JM, Pogrzeba
350 M, Krzyzak J (2005): The use of indigenous plant species and calcium phosphate for
351 the stabilization of highly metal-polluted sites in southern Poland. Plant and Soil 273,
352 291-305
353 [8] Chen Z, Sheng X, He L, Huang Z, Zhang W (2013): Effects of root inoculation with
354 bacteria on the growth, Cd uptake and bacterial communities associated with rape
355 grown in Cd-contaminated soil. Journal of Hazardous Materials 244–245, 709-717
356 [9] Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011): Plant growth promoting
357 rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils.
358 Biotechnology Advances 29, 248-258
359 [10] Abou-Shanab RA, Ghanem K, Ghanem N, Al-Kolaibe A (2008): The role of bacteria
360 on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated
361 soils. World Journal of Microbiology and Biotechnology 24, 253-262
362 [11] Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J (2009): Exploiting
363 plant–microbe partnerships to improve biomass production and remediation. Trends in
364 Biotechnology 27, 591-598
365 [12] Zhuang X, Chen J, Shim H, Bai Z (2007): New advances in plant growth-promoting
366 rhizobacteria for bioremediation. Environment International 33, 406-413

[13] Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009): Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. Journal of Applied Microbiology 107, 1687-1696

[14] Glick B, Cheng Z, Czarny J, Duan J (2007): Promotion of plant growth by ACC deaminase-producing soil bacteria. European Journal of Plant Pathology 119, 329-339

[15] Beneduzi A, Ambrosini A, Passaglia LMP (2012): Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genetics and Molecular Biology 35, 1044-1051

[16] Tak H, Ahmad F, Babalola OO (2013): Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. In: Whitacre DM (Ed.), Reviews of environmental contamination and toxicology Volume 223. Springer New York, 33-52

[17] Nogueira MA, Nehls U, Hampp R, Poralla K, Cardoso E (2007): Mycorrhiza and soil bacteria influence extractable iron and manganese in soil and uptake by soybean. Plant and Soil 298, 273-284

[18] Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009): Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. Soil Biology and Biochemistry 41, 154-162

[19] Schütze E, Klose M, Merten D, Nietzsche S, Senftleben D, Roth M, Kothe E (2014): Growth of streptomycetes in soil and their impact on bioremediation. Journal of Hazardous Materials 267, 128-135

[20] Vamerali T, Bandiera M, Mosca G (2009): Field crops for phytoremediation of metal-contaminated land. A review. Environmental Chemistry Letters 8, 1-17

[21] Büchel G, Bergmann H, Ebena G, Kothe E (2005): Geomicrobiology in remediation of mine waste. Chemie Der Erde-Geochemistry 65, 1-5

392 [22] Zeien H, Brümmer G (1989): Chemische Extraktionen zur Bestimmung von
393 Schwermetallbindungsformen in Böden. Mitteilungen der Deutschen Bodenkundlichen
394 Gesellschaft 59, 505-510
395 [23] Schmidt A, Haferburg G, Sineriz M, Merten D, Büchel G, Kothe E (2005): Heavy
396 metal resistance mechanisms in actinobacteria for survival in AMD contaminated soils.
397 Chemie Der Erde-Geochemistry 65, 131-144
398 [24] Schmidt A, Haferburg G, Schmidt A, Lischke U, Merten D, Ghergel F, Büchel G,
399 Kothe E (2009): Heavy metal resistance to the extreme: *Streptomyces* strains from a
400 former uranium mining area. Chemie Der Erde-Geochemistry 69, 35-44
401 [25] Amoroso MJ, Schubert D, Mitscherlich P, Schumann P, Kothe E (2000): Evidence for
402 high affinity nickel transporter genes in heavy metal resistant *Streptomyces* spec.
403 Journal of Basic Microbiology 40, 295-301
404 [26] Clemente R, Walker DJ, Bernal MP (2005): Uptake of heavy metals and As by
405 *Brassica juncea* grown in a contaminated soil in Aznalcóllar (Spain): The effect of soil
406 amendments. Environmental Pollution 138, 46-58
407 [27] Ebbs SD, Kochian LV (1998): Phytoextraction of zinc by oat (*Avena sativa*), barley
408 (*Hordeum vulgare*), and Indian mustard (*Brassica juncea*). Environmental Science and
409 Technology 32, 802-806
410 [28] Haferburg G, Reinicke M, Merten D, Büchel G, Kothe E (2007): Microbes adapted to
411 acid mine drainage as source for strains active in retention of aluminum or uranium.
412 Journal of Geochemical Exploration 92, 196-204
413 [29] Langella F, Grawunder A, Stark R, Weist A, Merten D, Haferburg G, Büchel G, Kothe
414 E (2014): Microbially assisted phytoremediation approaches for two multi-element
415 contaminated sites. Environmental Science and Pollution Research 21, 6845-6858
416 [30] Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000): Role of plants,
417 mycorrhizae and phytochelators in heavy metal contaminated land remediation.
418 Chemosphere 41, 197-207

[31] Bonfante P, Genre A (2010): Mechanisms underlying beneficial plant - fungus interactions in mycorrhizal symbiosis. Nature Communications 1:48

[32] Jasinskas A, Simonaviciute R, Siaudinis G, Liaudanskiene I, Antanaitis S, Arak M, Olt J (2014): The assessment of common mugwort (*Artemisia vulgaris* L.) and cup plant (*Silphium perfoliatum* L.) productivity and technological preparation for solid biofuel. Agriculture 101, 19-26

[33] Neagoe A, Stancu P, Nicoară A, Onete M, Bodescu F, Gheorghe R, Iordache V (2014): Effects of arbuscular mycorrhizal fungi on *Agrostis capillaris* grown on amended mine tailing substrate at pot, lysimeter, and field plot scales. Environmental Science and Pollution Research 21, 6859-6876

[34] Toler HD, Morton JB, Cumming JR (2005): Growth and metal accumulation of mycorrhizal sorghum exposed to elevated copper and zinc. Water, Air, and Soil Pollution 164, 155-172

[35] Adriano-Anaya ML, Salvador-Figueroa M, Ocampo JA, Garcia-Romera I (2006): Hydrolytic enzyme activities in maize (*Zea mays*) and sorghum (*Sorghum bicolor*) roots inoculated with *Gluconacetobacter diazotrophicus* and *Glomus intraradices*. Soil Biology and Biochemistry 38, 879-886

[36] Schreiner RP, Koide RT (1993): Streptomycin reduces plant response to mycorrhizal infection. Soil Biology and Biochemistry 25, 1131-1133

[37] Lasat MM (2002): Phytoextraction of toxic metals: A review of biological mechanisms. Journal of Environmental Quality 31, 109-120

[38] Zhang X, Xia H, Li Z, Zhuang P, Gao B (2010): Potential of four forage grasses in remediation of Cd and Zn contaminated soils. Bioresource Technology 101, 2063-2066

[39] Moreira H, Marques AGC, Franco A, Rangel ASS, Castro PL (2014): Phytomanagement of Cd-contaminated soils using maize (*Zea mays* L.) assisted by plant growth-promoting rhizobacteria. Environmental Science and Pollution Research 21, 9742-9753

446 [40] Usman ARA, Mohamed HM (2009): Effect of microbial inoculation and EDTA on the
447 uptake and translocation of heavy metal by corn and sunflower. Chemosphere 76, 893-
448 899

4 Discussion

Phytoremediation is a cost-effective and sustainable alternative for the treatment of heavy metal-contaminated soil at large scale. This emerging strategy uses green plants as solar-driven pumps to remove, transfer, or stabilize metal pollutants from contaminated environments (Chaney et al. 1997, Prasad 2004). In order to remediate sites contaminated by heavy metals phytoextraction and phytostabilization are the most reliable approaches. Phytoextraction is based on the use of high-biomass, metal-accumulating plants that can extract and accumulate metals from the soil into aboveground plant compartments (Garbisu and Alkorta 2001), while phytostabilization or phytorestoration reduces the risk of metal contaminants in soil system by formation of insoluble metal species resulting in a decreased metal mobility and bioavailability (Kucharski et al. 2005). The phytoremediation efficiency depends on both accumulation capacity by a particular plant species and biomass productivity. Fast-growing, high biomass producing crop species have been shown to be promising candidates for remediation of multi-elemental contaminated sites (Ciura et al. 2005, Farrag et al. 2012, Zhuang et al. 2007). The application of metal-resistant soil microorganisms can affect plant growth and metal uptake and thus, positively influence phytoremediation purposes. This can change the availability of nutrients to plants, but also lower metal toxicity within plants or soil, e.g., by releasing metal chelators like siderophores (Khan 2005). Additionally, microbial activity within rhizosphere can alter soil properties (e.g., pH and Eh) and make metals available for plant uptake (Abou-Shanab et al. 2006) or reduce their mobility in soil, thus limiting metal leaching into groundwater (Haferburg et al. 2007). Hence, interactions between soil microorganisms and plant roots within the rhizosphere play a crucial role in phytoremediation of heavy metal-contaminated soils. This work focused on the impact of heavy metal-resistant streptomycetes and the arbuscular mycorrhizal fungus *Rhizophagus irregularis* on plant growth and metal removal of high biomass producing crop species growing in multi-metal contaminated soil. The results of this study emphasize the potential of microbial inoculation on plant growth promotion and supporting remediation of contaminated soil by metal-tolerant crop plants.

4.1 Impact of microbial inoculation on plant performance

The biomass productivity of the grasses *Andropogon gerardii* and *Sorghum bicolor*, and the herbaceous crop plant *Silphium perfoliatum* on metal-contaminated substrate was evaluated under greenhouse conditions. The tested plant species achieved a satisfactory

growth without symptoms of metal toxicity thus, indicating their tolerance to several heavy metals (Banks et al. 1994, Jadia and Fulekar 2008). The highest biomass production was shown by *S. bicolor*, followed by *S. perfoliatum* and *A. gerardii* with the lowest biomass yield. *Silphium* genus species are usually characterized as high yielding crops with high potential for renewable energy purposes (Gansberger et al. 2015, Voigt et al. 2012). Biomass yields of these perennial plants have been reported to be equal to or 34 % higher than forage sorghum yields (Vetter et al. 2007). The relatively low dry matter yields of *S. perfoliatum* in contrast to *Sorghum* plants after 12 weeks could be explained by its typical growth dynamics, involving increasing biomass productivity from the second-year vegetation onwards (Jasinskas et al. 2014, Siaudinis et al. 2012).

Soil-borne bacteria or mycorrhizal fungi are vital components of the rhizosphere, involved in plant interactions and capable to promote plant growth by colonizing root surfaces. These beneficial soil bacteria, usually referred to as plant growth promoting bacteria (PGPB), can facilitate plant biomass production in both ways, directly or indirectly (Kloepper et al. 1989). Mechanisms of plant growth promotion are (1) nutrient acquisition by providing micro- and macro-nutrients including nitrogen, phosphorous and iron; (2) synthesis of plant growth promoting compounds or phytohormones such as auxins, cytokinins and gibberellins; (3) exerting ACC deaminase activity; (4) acting as biocontrol agents by competing with plant pathogens and inhibiting their activity; and (5) reducing metal phytotoxicity by immobilization of metals via sorption or accumulation processes (Ahmad et al. 2008, Beneduzi et al. 2012, Crowley et al. 1991, Glick et al. 2007, Tanimoto 2005, Weyens et al. 2009, Zaidi et al. 2009). At the same time, they also have been found to act as biofertilizer by enhancing soil fertility and thus, increase crop biomass productivity as well as sustainability of agriculture (Singh et al. 2011). Their physiological properties not only allow for improved plant growth but also for enhanced remediation of metalliferrous soils by PGPB inoculated metal-tolerant plants (Dary et al. 2010, Glick 2003, Kuffner et al. 2008). Thereby, plant growth promotion is mainly influenced by secretion of IAA, siderophore production and/or presence of the enzyme ACC deaminase (Glick 2010). In most cases not only one, but several of these activities are involved.

Despite beneficial traits of rhizosphere bacteria on plant growth symbiosis with arbuscular mycorrhizal fungi (AMF) can also contribute to plant health and growth. In the symbiosis, plants profit from enhanced nutrient acquisition (e.g., P, K, Zn, N and water) through extrametrical fungal hyphae, which increases root surface area of plants, resulting in an improved plant performance and protection against excess metals (Hildebrandt et al. 2007, Smith and Smith 2011). In turn, AM fungi obtain carbohydrates, mainly as glucose or sucrose, from their host plants. Arbuscular mycorrhizae have also been reported in

heavy metal contaminated soils (Leyval et al. 1997, Shetty et al. 1995, Tonin et al. 2001, Turnau et al. 2008). It has been found that different species of arbuscular mycorrhizal fungi, mostly of the genus *Glomus* or *Gigaspora*, colonize roots of metallophytes including members of the family Brassicaceae and thus, contribute to plant tolerance to heavy metal stress (Hildebrandt et al. 2002, Orłowska et al. 2002, Raman et al. 1993). In nutrient poor, metal-rich habitats they are largely responsible for a successful establishment of plants. Therefore, creating a stabilizing vegetation cover on soils rich in toxic metals is potentially useful for soil remediation (Doubková et al. 2012, Hildebrandt et al. 2007).

Exceeding metal concentrations in soil adversely affect soil microbial communities by reduction of total microbial biomass, decrease of specific populations or changing of microbial community structure (Giller et al. 1998, Hiroki 1992, Sobolev and Begonia 2008). In order to facilitate metal removal by plants applied soil bacteria and mycorrhizal fungi need to be resistant themselves against high concentrations of heavy metals in soil environment. To overcome heavy metal stress, soil microorganisms including PGPB and AM fungi have evolved several resistance mechanisms. In general, such mechanisms include (a) extrusion of metal ions by high affinity efflux transporters, (b) intracellular compartmentation or sequestration by metal-binding proteins or other ligands, (c) enzymatic transformation and detoxification of metals to a less toxic form, (d) extracellular chelation and complexation by excreting of chelating compounds and (e) extracellular sequestration by sorption to the cell envelope (Bruins et al. 2000, Nies 1999, Silver and Phung 1996). It has been found that Gram-positive, aerobic soil bacteria, specifically streptomycetes, combine different mechanisms of metal resistance to both survive and promote plant growth in highly contaminated habitats (Amoroso et al. 2000, Haferburg and Kothe 2007). The process of biosorption – binding of metals to the cell surface and other cell wall components – is an extracellular resistance mechanism predominantly in Gram-positive bacteria, including streptomycetes. Different *Streptomyces* strains have been found to adsorb toxic metals like U, Al, Pb, Cd and Cr in high amounts onto their cell walls, also as dead microbial biomass (Amoroso et al. 2001, Haferburg et al. 2007).

The extracellular chelation of metals by siderophore excretion could be shown for heavy metal-resistant streptomycetes, particularly in the presence of high metal concentrations. The strain *Streptomyces acidiscabies* E13 was shown to produce three different siderophores of the hydroxamate type at the same time in the presence of elevated Ni concentrations (Dimkpa et al. 2008). In addition, the production of soluble brownish pigments (melanin-like compounds) has been described for several *Streptomyces* isolates under heavy metal stress. These excreted secondary metabolites have been suggested to play a role in heavy metal resistance due to their metal-chelating properties and by acting

as a sink for produced reactive oxygen species (Haferburg and Kothe 2007, Riley 1997). For instance, two heavy metal-resistant *S. mirabilis* strains isolated from a former uranium mining area have been found to produce a melanin-like brown pigment on nickel-enriched complex media (Schmidt et al. 2009). Furthermore, the ability to transform certain metals into less toxic forms by metal reduction has been reported for some actinobacteria, especially streptomycetes. For three copper-resistant actinobacteria strains, belonging to the genus *Streptomyces*, the reduction of Cu(II) to Cu(I) by extracellular cupric reductase activity has been described (Albarracin et al. 2008b, Dávila Costa et al. 2011). Another intracellular resistance mechanism which has been described for metal-resistant streptomycetes includes the expression of superoxide dismutases (SOD). These catalytic enzymes are involved in detoxification of reactive oxygen species, but are also responsible for incorporating specific metal ions into their active center in the presence of high metal levels and thus, enhance metal resistance (Kim et al. 1996, Kothe et al. 2010, Schmidt et al. 2007). The differential expression of nickel (NiSOD) and iron (FeZnSOD) – containing superoxide dismutases in heavy metal-supplemented medium could be shown for the highly metal-resistant *Streptomyces* strain E13, particularly at high concentrations of Ni (Schmidt et al. 2007). Additionally, intracellular sequestration mediated by small metal-binding proteins such as metallothioneins (MTs), low-molecular weight peptides with a high cysteine and/or hystidine content, have been reported mainly in cyanobacteria and proteobacteria (Blindauer et al. 2002, 2007). Within Gram-positive actinobacteria, only a small copper-binding cysteine-rich protein in *Mycobacterium tuberculosis* has previously been identified (Gold et al. 2008). However, the screening of 73 actinobacterial genomes for small cysteine- and hystidine-rich peptides (≤ 100 amino acids) revealed 103 putative metallothioneins and metallohistins (Schmidt et al. 2010). Active transport or efflux systems to reduce high intracellular metal concentrations are generally plasmid-encoded and represent one of the largest metal resistance mechanisms (Bruins et al. 2000, Nies 2003). Most of the inducible, high affinity membrane transport systems with high substrate specificity belong to the ATP-binding cassette (ABC) superfamily or to the major facilitator superfamily (MFS), using ATP hydrolysis or chemiosmotic gradient as energy sources (Nies and Silver 1995). Numerous efflux transporter systems have been described for Gram-negative bacteria and yeast (Jungwirth and Kuchler 2006, Moreira et al. 2004, Nikaido 1996). For instance, in the Gram-negative bacterium *Ralstonia eutropha* resistance against cobalt, zinc and cadmium (Czc) is mediated by the Czc system, a proton-motive forced transenvelope transporter (Nies 1999). On the contrary, in actinobacteria ABC-type efflux transporters are predominantly responsible for antibiotic resistance. However, there is evidence for high affinity transporter system in *Streptomyces* strains, isolated from metal-contaminated soil (Amoroso et al. 2000).

Moreover, 56 genes encoding for efflux pumps could be identified in *S. coelicolor* A3(2) by transcriptome analysis (Kim et al. 2008).

The mechanisms employed by soil fungi to tolerate heavy metals are similar to those involved in bacterial metal resistance. Namely, extracellular complexation of heavy metals by cell wall adsorption, binding to fungal ligands and specific proteins including metallothioneins/phytochelatins and glutathione as well as active exclusion mediated by efflux transporters or intracellular sequestration in the vacuole compartment (Hall 2002, Jentschke and Godbold 2000, Pocsi et al. 2004). For example, a metallothionein gene was found to be expressed in response to oxidative stress in *Rhizophagus irregularis* (Gonzalez-Guerrero et al. 2007) or under exposure to Zn high transcript levels of a putative Zn transporter gene (GintZnT1), belonging to the cation diffusion facilitator family (CDF), were reported (Gonzalez-Guerrero et al. 2005).

In our greenhouse experiment both multi-metal resistant streptomycetes, isolated from a former uranium mining site, and the arbuscular mycorrhizal fungus *Rhizophagus irregularis* were applied to promote growth of selected plant species in contaminated substrate. After 12 weeks, the influence of microbial application on biomass productivity of the two grasses *A. gerardii* and *S. bicolor* as well as the herb *S. perfoliatum* was evaluated by measuring shoot dry weight. An effect of microbial inoculation on shoot biomass was seen for all plant species, particularly for plants amended with *Streptomyces* strains. The observed promoting impact of the inoculated streptomycetes on plant performance in metalliferrous soil is in line with earlier studies (Dimkpa et al. 2008, Dimkpa et al. 2009b, Schütze et al. 2014).

The beneficial effects of streptomycetes on plants roughly comprise plant growth promoting activities and plant disease suppression. In the process of growth promotion, the production of phytohormones plays an important role. These plant growth regulators contribute to diverse physiological processes including quiescence and seed germination, root formation, fruit ripening, and protect plants from environmental stress (Tsavkelova et al. 2006). A large number of *Streptomyces* strains were shown to produce auxins, gibberellins and cytokinin-like substances to stimulate seed germination and promote growth of different crop plants (Aldesuquy et al. 1998, Postolachi et al. 2013, Postolaky et al. 2012). Another important mechanism utilized by streptomycetes to facilitate plant growth and development is the lowering of ethylene levels by deamination of 1-aminocyclopropane-1-carboxylic acid (ACC) the immediate precursor of ethylene in plants (Arunachalam Palaniyandi et al. 2013, El-Tarabily 2008). Streptomycetes have also been found to synthesize an extraordinary variety of extracellular enzymes like agarases, proteases, amylases, cellulases, lipases, chitinases and phosphatases to degrade

complex organic and inorganic compounds (Schrempf et al. 2011). The secretion of such secondary metabolites allows streptomycetes to solubilize nutrients from a wide range of carbohydrates, alcohols, aromatic compounds or phosphates available in soil as complex or insoluble forms and thus, support plants to overcome nutrient deficiency in stressful environments (Chater et al. 2010, Crawford 1978, Schrempf 2001, Yang and Wang 1999). Other plant growth promoting features of some members of the genus *Streptomyces* include the fixation of nitrogen (Gtari et al. 2012, Pankratov and Dedysh 2009) and the solubilization of both organic and inorganic forms of phosphorous (Gupta et al. 2010). For instance, thermophilic *S. thermoautotrophicus* was found to fix nitrogen when growing chemolithoautotrophically under aerobic conditions at 65 °C (Gadkari et al. 1992, Ribbe et al. 1997). Several *Streptomyces* strains, isolated from Moroccan phosphate mines, have been found to solubilize rock phosphate by siderophore production and therefore stimulate biomass production of wheat plants (Hamdali et al. 2008a, 2008b). Whereas in another study, strains of *Streptomyces* isolated from wheat roots were shown to release soluble phosphate from buffered, phosphate-containing media by acidification through excretion of organic acids such as malate (Jog et al. 2014). In addition, the secretion of low-molecular mass iron chelators such as siderophores by streptomycetes has been found to overcome Fe deficiency of plants by siderophore-mediated iron uptake but also lower metal toxicity by forming metal complexes and thus, promote plant growth in heavy metal-contaminated soil (Dimkpa et al. 2008, 2009a, 2009b). Actinobacteria, especially streptomycetes, also exhibit immense biocontrol action against a wide range of phytopathogens by direct or indirect mechanisms. Direct antagonistic mechanisms include production of antibiotics such as cycloheximide and streptomycin to control fungal and bacterial plant diseases (de Lima Procópio et al. 2012, Waksman et al. 1946), cell wall-degrading extracellular enzymes with chitinolytic and glucanolytic activity (Chater et al. 2010, Gopalakrishnan et al. 2011, Nagpure et al. 2014, Sousa et al. 2008) and hyperparasitism on fungal pathogens (Palaniyandi et al. 2013, Tapio and Pohto-Lahdenperä 1991). Plant disease suppression by indirect antagonism involves competition for essential nutrients like e.g., iron through siderophore production (Macagnan et al. 2008) or induction of host resistance (Lehr et al. 2008, Zhao et al. 2012).

In this study, inoculation with the mycorrhizal fungus *Rhizophagus irregularis* has been found to promote growth of crop plants almost to the same extend as the *Streptomyces* strains. This effect is consistent with findings of Neagoe et al. (2014), who found that arbuscular mycorrhizal colonization of *Agrostis capillaris* in mine tailing substrate increased biomass production due to improved phosphorous availability and protecting plants from oxidative stress. In addition, consistent with Usman and Mohammed (2009), our results indicate that an established AMF symbiosis play a protective role against

excess metals and that mycorrhizal colonization contribute to acquisition of essential nutrients present at low concentrations in metal-contaminated soils, resulting in an improved plant growth. However, the efficiency of plant growth promotion and metal protection strongly depends on mycorrhizal fungi and heavy metals (Galli et al. 1994, Leyval et al. 1997). In contrast, dual inoculation of *R. irregularis* with *Streptomyces* strains had no visible effect on biomass production after 12 weeks of plant growth. However, several strains of *Streptomyces* have been found to function as so-called "mycorrhiza helper bacteria" and promote symbiosis between plants and mycorrhizal fungi, thus exhibiting plant growth beneficial effects (Abdel-Fattah and Mohamedin 2000, Becker et al. 1999, Schrey et al. 2012). For instance, *Streptomyces* strain AcH 505 has been reported to produce the fungal growth-promoting substance auxofuran which supports ectomycorrhizal formation (Riedlinger et al. 2006). Additionally, interactions between different *Streptomyces* isolates and the arbuscular fungus *Glomus mosseae* were found to promote mycorrhizal colonization of clover plants (Franco-Correa et al. 2010). Contrarily, streptomycetes can also inhibit VA mycorrhiza formation by production of antibiotics such as streptomycin and hence stimulating plant growth less than single inoculation (Krishna et al. 1982, Schreiner and Koide 1993). The obtained results revealed the potential of applied heavy metal-resistant *Streptomyces* strains and the arbuscular mycorrhizal fungus *R. irregularis* for multiple plant growth promoting activities.

4.2 Microbially mediated metal uptake into plant biomass

The use of metal-tolerant plant species to extract and translocate heavy metals to harvestable biomass or stabilize them within the rhizosphere provides a sustainable method for remediation of metal-contaminated soils. The efficiency of metal removal is mainly determined by two key factors: plant biomass and metal accumulation (Raskin et al. 1997). Plants exhibiting the potential to produce a significant amount of biomass while accumulating high metal concentrations thus removing large quantities of soil metals are desirable for phytoremediation purposes. In contrast to metal hyperaccumulator plants, fast-growing and high biomass producing crop species or metal-tolerant grasses can represent a potential alternative for removing heavy metals from contaminated soils by compensating lower shoot metal concentration with greater biomass productivity (Ebbs and Kochian 1998, Meers et al. 2005, Vamerali et al. 2009). The high biomass C_4 grass *Sorghum bicolor* has been reported to tolerate and accumulate high levels of Cd, Cu, Pb and Zn in the shoots and is considered to be one of the most drought resistant energy crop (Corredor et al. 2009, Zhuang et al. 2009). Similarly, the prairie grass *Andropogon gerardii* has also been found to be tolerant to soil metal contamination and to be adapted

on soils with low nutrients and limited water availability (Banks et al. 1994, Schultz et al. 2001). In addition, the herbaceous plant *Silphium perfoliatum*, count as a drought-tolerant crop, has been broadly investigated as high biomass producing forage or energy crop but so far little is known about its phytoremediation potential (Franzaring 2014, Siaudinis et al. 2012).

Since heavy metal uptake by plants does not only depend on metal bioavailability in soil, plant biomass and metal tolerance, we assessed whether inoculation with metal-resistant streptomycetes and the usage of arbuscular mycorrhiza affected metal removal. In our study, harvestable biomass of crop plants exclusively consisted of the aboveground parts and thus, metal concentrations in plant roots were not considered. Metal accumulation into shoot biomass differed significantly between tested plant species regardless of treatment. Highest concentrations of all tested metals were taken up by *Silphium* plants, while the grass *A. gerardii* was found to accumulate similar amounts of Al and U, respectively. In the literature, comprehensive studies on metal remediation potential of *S. perfoliatum* are still rare. In a study by Zhang et al. (2010), it could be shown, that Cd and Zn were predominantly accumulated in the roots of *S. perfoliatum*, suggesting that this plant could be suitable for stabilization of Cd/Zn contaminations. In our experiments, microbial inoculation had variable effects on metal uptake by tested plant species grown in highly contaminated substrate. For *S. perfoliatum*, this is the first report showing that microbial application led to a decreased metal uptake into shoot biomass. On the contrary, effects of metal-resistant streptomycetes and mycorrhizal fungus contributing to metal accumulation were clearly seen for the two grasses *A. gerardii* and *S. bicolor*. The addition of *Streptomyces* strains significantly increased concentrations of Co and Ni in shoots of sorghum plants. The effects seen with inoculation compare well with findings reported by Abou-Shanab et al. (2008), who found that bacterial inoculation of *S. bicolor* growing on soil with high metal contents led to an increase Cu and Cr accumulation into plant shoots compared to non-inoculated controls. Additionally, Duponnois et al. (2006) showed that bacterial addition significantly improved Cd uptake and translocation into shoots by sorghum plants under glasshouse conditions. The inoculation with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* slightly increased Cd and Zn concentrations in shoots of *S. bicolor*, while for the other metals a reduction of shoot metal uptake was observed.

Our results are consistent with those obtained by Toler et al. (2005), who showed that mycorrhizal inoculation increased metal accumulation by *Sorghum* plants exposed to elevated heavy metal concentrations. Moreover, Guo et al. (2013) also showed that application of different *Glomus* species on *S. bicolor* grown in mine tailing substrates

could either increase shoot metal concentrations or mostly, decrease plant heavy metal uptake. In case of *Andropogon gerardii*, inoculation with streptomycetes and/or mycorrhiza had different effects on shoot metal concentrations, showing that metal uptake is highly influenced by microbial treatment and metal contaminant. For instance, we observed that inoculation with the different *Streptomyces* strains resulted in both increased and decreased metal concentrations in plant shoots, while presence of mycorrhizal fungus mostly reduced translocation of metals into shoot biomass. Our findings are consistent with those reported in previous investigations (Banks et al. 1994, Shetty et al. 1994), showing that addition of bacteria could enhance metal uptake but without affecting metal concentrations in shoots whereas arbuscular mycorrhizal infection of *Andropogon* roots was found to significantly reduce metal uptake and heavy metal concentrations in plant shoots. Shetty et al. (1994) showed that inoculation of *A. gerardii* with soil bacteria resulted in increased Zn levels in shoots and that combined inoculation with mycorrhiza and other soil microbes led to higher shoot metal concentrations than mycorrhiza alone. In contrast, for *Andropogon virginicus* it was found that AMF reduce uptake and translocation of toxic Al concentrations, suggesting that mycorrhizal association contribute to Al resistance of host plants (Cumming and Ning 2003).

In our study, we could show that the application of metal-resistant soil microorganisms can contribute to metal extraction as well as stabilization in soil by high biomass producing crop species. Moreover, our study not only confirmed the suitability of the *S. perfoliatum* for phytoextraction but also highlighted the metal stabilization potential of the two grasses *A. gerardii* and *S. bicolor* in metal-contaminated soil.

4.3 Factors influencing metal mobility in soil

Since the total metal content in soil is a poor indicator of the actual metal concentration in soil solution, the bioavailable fraction is regarded as main risk of a possible contamination of both ground water and food chain. Metal solubility highly depends on soil characteristics including soil pH and the degree of complexation with soluble ligands (Norvell 1984, Violante et al. 2010). Heavy metals in soil solution can exist as either free (uncomplexed) metal ions, soluble metal complexes with various soil ligands or be associated with mobile inorganic and organic colloidal material (Ramos et al. 1994). Metals readily available for plant uptake are those that exist as soluble metal compounds in soil solution or are easily desorbed or solubilized by plant root exudates. Hence, effective metal removal by plants is dependent on high metal bioavailability to achieve considerable uptake into plant shoots. Plants can affect metal availability in soil solution either directly or indirectly. Direct mechanisms comprise the extraction of water and

nutrients or excretion of protons, inorganic ions and organic acids by plant roots. The release of root exudates may alter the chemical environment of the rhizosphere and therefore can increase or decrease concentration of metal ions in soil solution (Kim et al. 2010, Quartacci et al. 2009, Tao et al. 2004). Also, uptake of competing ions by plant roots can affect soil metal concentrations indirectly. In addition, the formation of macrospores that may act as preferential flow paths and the stimulation of microbial activity are other indirect effects of plant roots and root exudates influencing metal mobility (Albrecht et al. 2002, Barber and Lynch 1977, Nannipieri et al. 2008).

Soil microorganisms have also been shown to strongly influence metal bioavailability by altering solubility and mobility of heavy metals in soil through a variety of mobilization or immobilization processes (Gadd 2000, Jing et al. 2007, Ma et al. 2011, Schütze and Kothe 2012). For instance, rhizosphere bacteria and mycorrhizal fungi can alter metal availability by excretion of various metabolites including biosurfactants (Braud et al. 2006, Mulligan et al. 2001), siderophores (Dimkpa et al. 2008, Dimkpa et al. 2009b, Glick 2003), organic compounds or other metal-binding biomolecules (Haferburg and Kothe 2007, Rózycki and Strzelczyk 1986).

In our study, the effect of microbial inoculation was also evaluated in terms of soil metal bioavailability after plant growth in contaminated substrate. A significant reduction of initial bioavailable fractions of Sr could be observed for *A. gerardii* inoculated with streptomycetes, while bacterial addition increased Cu availability in soil planted with *S. perfoliatum.* Heavy metal-resistant streptomycetes have been shown to alter metal bioavailability through specific mechanisms involved in heavy metal resistance (Haferburg and Kothe 2007, Kothe et al. 2010). Gram-positive Actinobacteria, specifically streptomycetes have been reported to immobilize metals by either adsorption to cell wall mass (Rho and Kim 2002, Sineriz et al. 2009), release of extracellular chelating compounds like melanins or siderophores (Chater et al. 2010, Dimkpa et al. 2008) or intracellular accumulation (Albarracin et al. 2008a, Polti et al. 2011). In line with our results, Schütze et al. (2014) found that living biomass of metal-resistant *S. mirabilis* P16B-1 significantly decreased mobile fractions of different heavy metals in soil and effectively counteract metal mobilization by sorghum plants. On the contrary, numerous studies have shown bacterial-induced metal mobilization attributed to excretion of secondary metabolites including siderophore, organic acids and biosurfactants (Braud et al. 2009, Braud et al. 2006, Kuffner et al. 2010, Li et al. 2010, Ma et al. 2009, Marques et al. 2013, Rajkumar et al. 2010). In our experiment, inoculation with mycorrhizal fungus led to a significant reduction of soluble Cu for *S. bicolor* and Co for *Andropogon* plants, while double inoculation was found to decrease Co mobility in case of *S. perfoliatum*. These

findings are consistent with earlier studies (Banks et al. 1994, Schindler et al. 2012). For instance, Banks et al. (1994) showed that addition of either mycorrhizal fungi or a mixture of bacteria and mycorrhiza significantly reduced Zn concentration in leachates from mine tailings in comparison to unamended *Andropogon* plants. Similarly, Cumming and Ning (2003) reported a remarkably decrease of Al concentrations in leachates collected from mycorrhizal plants. In addition, metal tolerant arbuscular mycorrhizal fungi have been shown to immobilize heavy metals by changing metal speciation or complexation reactions in the rhizosphere and thus reducing their bioavailability in soil solution (Huang et al. 2005, Janouskova et al. 2006, Joner et al. 2000). Despite microbial impact on metal mobility, a mobilization of bioavailable Al and Ni occurred for untreated *Andropogon* and *Silphium*, suggesting that release of root exudates by plants could also play an important role in regulating metal phytoavailability (Maqsood et al. 2011, Nigam et al. 2001, Quartacci et al. 2009).

4.4 Application of phytoremediation in the field

The majority of experiments investigating the impact of soil microorganisms on phytoremediation performance of high biomass crop plants are at pot or lysimeter scale. Both small– and mesoscale experiments are extremely useful as a means to carry out comparable and reproducible studies on plant metal removal efficacy under controlled conditions. However, data from pot experiments cannot be directly extrapolated to real field sites and therefore should be assessed critically. Successful phytoremediation under field conditions not only depends on site-specific factors such as (1) local geological and hydrological situation; (2) climatic conditions (mostly temperature and rain); (3) soil characteristics (e.g., pH, Eh, nutrient availability, water table); (4) spatial distribution of metal contaminants and hot spot formations; and (5) soil metal mobility and availability but also selection of crop species, rotation management and field irrigation practices have to be considered for field application (Gary 1999, Haslmayr et al. 2014, Keller 2006). The successful transfer of phytoremediation from the laboratory scale to the field provides a crucial step in the commercial application of this green technique for decontamination of sites contaminated by heavy metals (McIntyre 2003). At present, only few studies intended to evaluate the phytoremediation potential of high biomass producing crop species, like *S. bicolor*, at metal-contaminated field sites (Fellet et al. 2007, Marchiol et al. 2007, Zhuang et al. 2009, Zhuang et al. 2007).

In our study, the impact of microbial amendments on biomass productivity and metal extraction by *S. bicolor* growing in metal-contaminated soil was evaluated at pot scale and under field conditions. An effect of microbial inoculation on biomass production of *S.*

bicolor was seen in plants treated with a combination of metal-resistant streptomycetes and mycorrhiza at pot scale and for plants growing in the nonamended control plot at the field site. The application of microbial inocula enhanced metal accumulation into shoot biomass of *S. bicolor*, Sr in particular, in greenhouse pots, while such an effect was not present for *Sorghum* grown in the field. In addition, the comparison of bioavailable soil fractions before and after plant growth showed that both plants and soil microbes strongly influenced bioavailability of heavy metals within soil rhizosphere. In this study, *Sorghum* plants demonstrated a different pattern and microbial application had variable effects on metal accumulation in field conditions compared to the pot experiments. Further, our work shows the necessity of using different experimental scales to assess the efficiency of microbially assisted phytoremediation. Our findings compare well with recent studies showing that the efficacy of phytoremediation can strongly differ between the investigated scales (Brunetti et al. 2011, Delorme et al. 2000, Farrag et al. 2012, Wernitznig et al. 2014). The different results between small-scale and large-scale experiments can be attributed to various factors, first and foremost due to the diversity of experimental systems, high spatial and climatic heterogeneity in the field, different physiological state of the plant, differences in substrate properties (water retention, compaction) or soil volume and watering conditions (natural rain versus regular irrigation). Further, the occurrence and diversity of invading weed species are other influencing factors in field phytoremediation. Moreover, the pot as closed system with limited volume prevents exchanges with other systems. The preparation of the potting substrate including air-drying and homogenization can modify the distribution of soluble metal fractions resulting in an enhanced metal phytoavailability and increased contact between plant roots, soil water and the soil matrix (Conesa et al. 2007, Neagoe et al. 2014, Nowack et al. 2004, Sprocati et al. 2014, Tlustos et al. 2006).

Although microbially supported phytoremediation shows promise as a useful tool for remediation of metal-contaminated soils (Abou-Shanab et al. 2008, Abou-Shanab et al. 2006, Langella et al. 2014, Sprocati et al. 2014), fundamental research for practical application in the field is still needed.

5 Abstract

Soil contamination by heavy metals as a result of increasing mining and industrial activities is a serious problem in many areas worldwide. Since heavy metals are not biodegradable, their remediation is of high importance. The clean-up of metal-contaminated soils by conventional methods is often cost- and energy-intensive, site destructive and technically limited to relatively small areas. Therefore, the use of plants to remediate heavy metal-contaminated sites, known as phytoremediation, represents a cost-effective and environmentally friendly alternative. In this work, greenhouse and field-scale experiments were performed in order to assess the effect of microbial inoculation on plant growth and metal remediation potential of high biomass producing crop species grown in multi-metal contaminated soil. The impact of microbial amendments on plant performance and metal extraction potential of *Sorghum bicolor* was investigated in pot experiments followed by field trials using metal-contaminated soil material from the test site Gessenwiese. Moreover, in a greenhouse study the effects of root colonization by the arbuscular-mycorrhizal fungus *Rhizophagus irregularis* and the application of heavy metal-resistant *Streptomyces* strains on metal mobilization/stabilization in soil and metal uptake by the grasses *Andropogon gerardii* and *Sorghum bicolor*, and the high biomass field crop *Silphium perfoliatum* were evaluated.

Biomass productivity and heavy metal uptake into plant shoots of *A. gerardii*, *S. bicolor* and *S. perfoliatum* were significantly influenced by the microbial treatments. The inoculation with metal-resistant streptomycetes and the usage of arbuscular mycorrhiza had variable effects on metal removal efficiency of tested plant species and was found to be plant and element specific. Both plant growth and microbial amendments could decrease soil metal bioavailability in pot experiments, while under field conditions no visible changes in metal mobility were detected. From this data it can be concluded that heavy metal-resistant streptomycetes and the mycorrhizal fungus *Rhizophagus irregularis* are able to promote plant growth, decrease heavy metal mobility in soil and at the same time facilitate metal accumulation into plant biomass. Thus, phytoremediation efficacy of high biomass crop plants grown on metal contaminated substrate could be enhanced.

6 Zusammenfassung

Die Verschmutzung des Bodens durch Schwermetalle in Folge zunehmender bergbaulicher und industrieller Aktivitäten stellt ein ernsthaftes Problem in vielen Teilen der Welt dar. Da Schwermetalle nicht biologisch abbaubar sind, ist ihre Entfernung von großer Bedeutung. Die Sanierung von schwermetallbelasteten Böden mittels konventionellen Methoden ist oft kostenintensiv und aufwändig, landschaftszerstörend sowie technisch auf relativ kleine Flächen begrenzt. Die Verwendung von Pflanzen zur Sanierung schwermetallkontaminierter Gebiete, auch als Phytoremediation bezeichnet, stellt somit eine kostengünstige und umweltschonende Alternative dar. Im Rahmen dieser Arbeit wurden Gewächshaus- und Feldversuche durchgeführt, um den Einfluss mikrobieller Inokulation auf das Pflanzenwachstum und Sanierungspotential hochproduktiver Nutzpflanzen in schwermetallbelasteten Bodensubstrat zu bewerten. Der Einfluss mikrobieller Zusätze auf das Wachstum und Potential zur Metallaufnahme von *Sorghum bicolor* wurde zuerst in Topfversuchen und anschließend in Freilandexperimenten mit schwermetallbelasteten Bodenmaterial des Testfeldes Gessenwiese untersucht. Darüber hinaus wurden die Auswirkungen der Wurzelkolonisierung durch den arbuskulären Mykorrhiza-Pilz *Rhizophagus irregularis* und die Zugabe schwermetallresistenter *Streptomyces*-Stämme auf die Schwermetallmobilisierung/-stabilisierung im Boden und Schwermetallaufnahme der Gräser *Andropogon gerardii* und *Sorghum bicolor* sowie der ertragreichen Biomassepflanze *Silphium perfoliatum* beurteilt.

Die Biomasseproduktivität sowie die Schwermetallaufnahme in die Biomassen der Pflanzen *A. gerardii*, *S. bicolor* und *S. perfoliatum* waren signifikant durch die mikrobiellen Behandlungen beeinflusst. Die Inokulation mit den schwermetallresistenten Streptomyceten und die Verwendung der arbuskulären Mykorrhiza hatte unterschiedliche Effekte auf die Effizienz der Schwermetallentfernung der eingesetzten Pflanzenarten und zeigte sich als pflanzen- und elementspezifisch. Das Pflanzenwachstum sowie die mikrobiellen Zusätze konnten in den Topfversuchen die Metallverfügbarkeit im Boden verringern, während diese Änderungen in der Schwermetallmobilität unter Freilandbedingungen nicht beobachtet wurden. Aus den erhaltenen Daten kann geschlussfolgert werden, dass die schwermetallresistenten Streptomyceten und der Mykorrhiza-Pilz *Rhizophagus irregularis* die Fähigkeit besitzen, das Pflanzenwachstum zu fördern, die Schwermetallmobilität im Boden zu reduzieren und gleichzeitig die Metallaufnahme in die Pflanzenbiomasse zu unterstützen. Demzufolge könnte die

Phytoremediationsleistung ertragreicher Kulturpflanzen in metallbelastetem Bodensubstrat gesteigert werden.

7 References

Abbas A, Edwards C (1989): Effects of metals on a range of *Streptomyces* species. Applied and Environmental Microbiology 55, 2030-2035

Abdel-Fattah GM, Mohamedin AH (2000): Interactions between a vesicular-arbuscular mycorrhizal fungus (*Glomus intraradices*) and *Streptomyces coelicolor* and their effects on sorghum plants grown in soil amended with chitin of brawn scales. Biology and Fertility of Soils 32, 401-409

Abedin MJ, Feldmann J, Meharg AA (2002): Uptake kinetics of arsenic species in rice plants. Plant Physiology 128, 1120-1128

Abou-Shanab RA, Ghanem K, Ghanem N, Al-Kolaibe A (2008): The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. World Journal of Microbiology and Biotechnology 24, 253-262

Abou-Shanab RAI, Angle JS, Chaney RL (2006): Bacterial inoculants affecting nickel uptake by *Alyssum murale* from low, moderate and high Ni soils. Soil Biology and Biochemistry 38, 2882-2889

Adriano-Anaya ML, Salvador-Figueroa M, Ocampo JA, Garcia-Romera I (2006): Hydrolytic enzyme activities in maize (*Zea mays*) and sorghum (*Sorghum bicolor*) roots inoculated with *Gluconacetobacter diazotrophicus* and *Glomus intraradices*. Soil Biology and Biochemistry 38, 879-886

Adriano DC, Wenzel WW, Vangronsveld J, Bolan NS (2004): Role of assisted natural remediation in environmental cleanup. Geoderma 122, 121-142

Ahemad M (2014): Remediation of metalliferous soils through the heavy metal resistant plant growth promoting bacteria: paradigms and prospects. Arabian Journal of Chemistry (*In Press*)

Ahemad M, Kibret M (2014): Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. Journal of King Saud University - Science 26, 1-20

Ahmad F, Ahmad I, Khan MS (2008): Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological Research 163, 173-181

Ahmed E, Holmström SJM (2014): Siderophores in environmental research: roles and applications. Microbial Biotechnology 7, 196-208

Akcil A, Koldas S (2006): Acid Mine Drainage (AMD): causes, treatment and case studies. Journal of Cleaner Production 14, 1139-1145

Albarracin VH, Winik B, Kothe E, Amoroso MJ, Abate CM (2008a): Copper bioaccumulation by the actinobacterium *Amycolatopsis* sp ABO. Journal of Basic Microbiology 48, 323-330

Albarracin VH, Avila AL, Amoroso MJ, Abate CM (2008b): Copper removal ability by *Streptomyces* strains with dissimilar growth patterns and endowed with cupric reductase activity. FEMS Microbiology Letters 288, 141-148

Albrecht A, Schultze U, Liedgens M, Fluhler H, Frossard E (2002): Incorporating soil structure and root distribution into plant uptake models for radionuclides: toward a more physically based transfer model. Journal of Environmental Radioactivity 59, 329-350

Aldesuquy HS, Mansour FA, Abo-Hamed SA (1998): Effect of the culture filtrates of *Streptomyces* on growth and productivity of wheat plants. Folia Microbiologica 43, 465-470

Ali H, Khan E, Sajad MA (2013): Phytoremediation of heavy metals – concepts and applications. Chemosphere 91, 869-881

Alloway BJ (1995): Soil processes and the behaviour of metals. In: Alloway BJ (Ed.), Heavy metals in soils. Blackie Academic and Professional, London, 11-37

Alloway BJ, Ayres DC (1998): Chemical principles of environmental pollution. Water, Air, and Soil Pollution 102, 216-218

Ames RN, Reid CPP, Ingham ER (1984): Rhizosphere bacterial population responses to root colonization by a vesicular arbuscular mycorrhizal fungus. New Phytologist 96, 555-563

Amoroso MJ, Schubert D, Mitscherlich P, Schumann P, Kothe E (2000): Evidence for high affinity nickel transporter genes in heavy metal resistant *Streptomyces* spec. Journal of Basic Microbiology 40, 295-301

Amoroso MJ, Castro GR, Duran A, Peraud O, Oliver G, Hill RT (2001): Chromium accumulation by two *Streptomyces* spp. isolated from riverine sediments. Journal of Industrial Microbiology and Biotechnology 26, 210-215

Anderson CWN, Brooks RR, Chiarucci A, LaCoste CJ, Leblanc M, Robinson BH, Simcock R, Stewart RB (1999): Phytomining for nickel, thallium and gold. Journal of Geochemical Exploration 67, 407-415

Arthur EL, Rice PJ, Rice PJ, Anderson TA, Baladi SM, Henderson KLD, Coats JR (2005): Phytoremediation—an overview. Critical Reviews in Plant Sciences 24, 109-122

Arunachalam Palaniyandi S, Yang SH, Damodharan K, Suh JW (2013): Genetic and functional characterization of culturable plant-beneficial actinobacteria associated with yam rhizosphere. Journal of Basic Microbiology 53, 985-995

Babich H, Stotzky G (1983): Developing standards for environmental toxicants: the need to consider abiotic environmental factors and microbe-mediated ecologic processes. Environmental Health Perspectives 49, 247-260

Baker AJM, Brooks RR (1989): Terrestrial higher plants which hyperaccumulate metallic elements - a review of their distribution, ecology and phytochemistry. Biorecovery 1, 81-126

Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994): The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resources, Conservation and Recycling 11, 41-49

Banks MK, Schwab AP, Fleming GR, Hetrick BA (1994): Effects of plants and soil microflora on leaching of zinc from mine tailings. Chemosphere 29, 1691-1699

Banks D, Younger PL, Arnesen R-T, Iversen ER, Banks SB (1997): Mine-water chemistry: the good, the bad and the ugly. Environmental Geology 32, 157-174

Barac T, Taghavi S, Borremans B, Provoost A, Oeyen L, Colpaert JV, Vangronsveld J, van der Lelie D (2002): Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nature Biotechnology 22, 583-588

Barber D, Lynch J (1977): Microbial growth in the rhizosphere. Soil Biology and Biochemistry 9, 305-308

Becker DM, Bagley ST, Podila GK (1999): Effects of mycorrhizal-associated streptomycetes on growth of *Laccaria bicolor*, *Cenococcum geophilum*, and

Armillaria species and on gene expression in *Laccaria bicolor*. Mycologia 91, 33-40

Beneduzi A, Ambrosini A, Passaglia LMP (2012): Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genetics and Molecular Biology 35, 1044-1051

Blindauer CA, Harrison MD, Robinson AK, Parkinson JA, Bowness PW, Sadler PJ, Robinson NJ (2002): Multiple bacteria encode metallothioneinsand SmtA-like zinc fingers. Molecular Microbiology 45, 1421-1432

Blindauer CA, Razi MT, Campopiano DJ, Sadler PJ (2007): Histidine ligands in bacterial metallothionein enhance cluster stability. Journal of Biological Inorganic Chemistry 12, 393-405

Blume HP, Brümmer GW, Horn R, Kandeler E, Kögel-Knabner I, Kretzschmar R, Stahr K, Wilke BM (2010): Lehrbuch der Bodenkunde. Spektrum Akademischer Verlag, Heidelberg

Boer AH, Prins HBA, Zanstra PE (1983): Bi-phasic composition of trans-root electrical potential in roots of *Plantago* species: involvement of spatially separated electrogenic pumps. Planta 157, 259-266

Bojarczuk K, Kieliszewska-Rokicka B (2010): Effect of ectomycorrhiza on Cu and Pb accumulation in leaves and roots of silver birch (*Betula pendula* Roth.) seedlings grown in metal-contaminated soil. Water, Air, and Soil Pollution 207, 227-240

Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB, Scheckel K (2014): Remediation of heavy metal(loid)s contaminated soils – to mobilize or to immobilize? Journal of Hazardous Materials 266, 141-166

Bonfante P (2010): Plant–fungal interactions in mycorrhizas. Encyclopedia of Life Science, John Wiley, London

Bonfante P, Genre A (2010): Mechanisms underlying beneficial plant – fungus interactions in mycorrhizal symbiosis. Nature Communications 1:48

Boukhalfa H, Crumbliss AL (2002): Chemical aspects of siderophore mediated iron transport. BioMetals 15, 325-339

Bowen HJM (1979): Environmental chemistry of the elements. Academic Press, London

Boyd RS, Martens SN (1994): Nickel hyperaccumulated by *Thlaspi montanum* var. montanum is acutely toxic to an insect herbivore. Oikos 70, 21-25

Boyd RS, Shaw JJ, Martens SN (1994): Nickel hyperaccumulation defends *Streptanthus polygaloides* (Brassicaceae) against pathogens. American Journal of Botany 81, 294-300

Boyd V (1996): Pint-sized plants pack a punch in fight against heavy metals. Environmental Protection 7, 38-39

Boyd RS, Jaffre T, Odom JW (1999): Variation in nickel content in the nickel hyperaccumulating shrub *Psychotria douarrei* (Rubiaceae) from New Caledonia. Biotropica 31, 403-410

Brady KU, Kruckeberg AR, Bradshaw HD (2005): Evolutionary ecology of plant adaptation to serpentine soils. Annual Review of Ecology Evolution and Systematics 36, 243-266

Braud A, Jézéquel K, Vieille E, Tritter A, Lebeau T (2006): Changes in extractability of Cr and Pb in a polycontaminated soil after bioaugmentation with microbial producers of biosurfactants, organic acids and siderophores. Water, Air, and Soil Pollution 6, 261-279

Braud A, Jezequel K, Bazot S, Lebeau T (2009): Enhanced phytoextraction of an agricultural Cr- and Pb-contaminated soil by bioaugmentation with siderophore-producing bacteria. Chemosphere 74, 280-286

Brooks RR, Robinson BH (1998): The potential use of hyperaccumulators and other plants for phytomining. In: Brooks RR (Ed.), Plants that hyperaccumulate heavy metals: their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. CAB International, Wallingford, 327-356

Brooks RR, Robinson BH, Howes AW, Chiarucci A (2001): An evaluation of *Berkheya coddii* Roessler and *Alyssum bertolonii* Desv. for phytoremediation and phytomining of nickel. South African Journal of Science 97, 558-560

Brown G (1954): Soil morphology and mineralogy. A qualitative study of some gleyed soils from North-West England. Journal of Soil Science 5, 145-155

Bruins MR, Kapil S, Oehme FW (2000): Microbial resistance to metals in the environment. Ecotoxicology and Environmental Safety 45, 198-207

Brunetti G, Farrag K, Rovira PS, Nigro F, Senesi N (2011): Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by *Brassica napus* from contaminated soils in the Apulia region, Southern Italy. Geoderma 160, 517-523

Brunner I, Luster J, Günthardt-Goerg MS, Frey B (2008): Heavy metal accumulation and phytostabilisation potential of tree fine roots in a contaminated soil. Environmental Pollution 152, 559-568

Büchel G, Bergmann H, Ebena G, Kothe E (2005): Geomicrobiology in remediation of mine waste. Chemie Der Erde-Geochemistry 65, 1-5

Burken JG, Schnoor JL (1997): Uptake and metabolism of atrazine by poplar trees. Environmental Science and Technology 31, 1399-1406

Chaney RL (1983): Plant uptake of inorganic waste. In: Parr JE, Marsh PB, Kla JM (Eds.), Land treatment of hazardous waste. Noyes Data Corp, Park Ridge, Il, USA, 50-76

Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997): Phytoremediation of soil metals. Current Opinion in Biotechnology 8, 279-284

Chater KF, Biro S, Lee KJ, Palmer T, Schrempf H (2010): The complex extracellular biology of *Streptomyces*. FEMS Microbiology Reviews 34, 171-198

Chen ZJ, Sheng XF, He LY, Huang Z, Zhang WH (2013): Effects of root inoculation with bacteria on the growth, Cd uptake and bacterial communities associated with rape grown in Cd-contaminated soil. Journal of Hazardous Materials 244–245, 709-717

Christensen TH, Kjeldsen P, Albrechtsen HJr, Heron G, Nielsen PH, Bjerg PL, Holm PE (1994): Attenuation of landfill leachate pollutants in aquifers. Critical Reviews in Environmental Science and Technology 24, 119-202

Chuan MC, Shu GY, Liu JC (1996): Solubility of heavy metals in a contaminated soil: effects of redox potential and pH. Water, Air, and Soil Pollution 90, 543-556

Ciura J, Poniedzialek M, Sekara A, Jedrszczyk E (2005): The possibility of using crops as metal phytoremediants. Polish Journal of Environmental Studies 14, 17-22

Clemente R, Walker DJ, Bernal MP (2005): Uptake of heavy metals and As by *Brassica juncea* grown in a contaminated soil in Aznalcóllar (Spain): the effect of soil amendments. Environmental Pollution 138, 46-58

Conesa HM, Robinson BH, Schulin R, Nowack B (2007): Growth of *Lygeum spartum* in acid mine tailings: response of plants developed from seedlings, rhizomes and at field conditions. Environmental Pollution 145, 700-707

Corredor DY, Salazar JM, Hohn KL, Bean S, Bean B, Wang D (2009): Evaluation and characterization of forage Sorghum as feedstock for fermentable sugar production. Applied Biochemistry and Biotechnology 158, 164-179

Crawford DL (1978): Lignocellulose decomposition by selected *Streptomyces* strains. Applied and Environmental Microbiology 35, 1041-1045

Crowley DE, Wang YC, Reid CPP, Szaniszlo PJ (1991): Mechanisms of iron acquisition from siderophores by microorganisms and plants. Plant and Soil 130, 179-198

Cumming JR, Ning J (2003): Arbuscular mycorrhizal fungi enhance aluminium resistance of broomsedge (*Andropogon virginicus* L.). Journal of Experimental Botany 54, 1447-1459

Cunningham SD, Berti WR, Huang JW (1995): Phytoremediation of contaminated soils. Trends in Biotechnology 13, 393-397

Cunningham SD, Anderson TA, Schwab AP, Hsu FC (1996): Phytoremediation of soils contaminated with organic pollutants. Advances in Agronomy 56, 55-114

Cunningham SD, Shann JR, Crowley DE, Anderson TA (1997): Phytoremediation of contaminated water and soil. Journal of the American Chemical Society 664, 2-17

Dary M, Chamber-Perez MA, Palomares AJ, Pajuelo E (2010): "In situ" phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. Journal of Hazardous Materials 177, 323-330

Dávila Costa J, Albarracín VH, Abate CM (2011): Cupric reductase activity in copper-resistant *Amycolatopsis tucumanensis*. Water, Air, and Soil Pollution 216, 527-535

de Lima Procópio RE, da Silva IR, Martins MK, de Azevedo JL, de Araújo JM (2012): Antibiotics produced by *Streptomyces*. The Brazilian Journal of Infectious Diseases 16, 466-471

Delorme TA, Angle JS, Coale FJ, Chaney RL (2000): Phytoremediation of phosphorus-enriched soils. International Journal of Phytoremediation 2, 173-181

Demchik MC, Sharpe WE (2001): Forest floor plant response to lime and fertilizer before and after partial cutting of a northern red oak stand on an extremely acidic soil in Pennsylvania, USA. Forest Ecology and Management 144, 239-244

Di Gregorio S, Lampis S, Vallini G (2005): Selenite precipitation by a rhizospheric strain of *Stenotrophomonas sp*. isolated from the root system of *Astragalus bisulcatus*: a biotechnological perspective. Environment International 31, 233-241

Dimkpa CO, Svatos A, Dabrowska P, Schmidt A, Boland W, Kothe E (2008): Involvement of siderophores in the reduction of metal-induced inhibition of auxin synthesis in *Streptomyces* spp. Chemosphere 74, 19-25

Dimkpa CO, Svatoš A, Merten D, Büchel G, Kothe E (2008): Hydroxamate siderophores produced by *Streptomyces acidiscabies* E13 bind nickel and promote growth in cowpea (*Vigna unguiculata* L.) under nickel stress. Canadian Journal of Microbiology 54, 163-172

Dimkpa CO, Weinand T, Asch F (2009): Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant, Cell and Environment 32, 1682-1694

Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009a): Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. Journal of Applied Microbiology 107, 1687-1696

Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009b): Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. Soil Biology and Biochemistry 41, 154-162

Doty SL (2008): Enhancing phytoremediation through the use of transgenics and endophytes. New Phytologist 179, 318-333

Doubková P, Suda J, Sudová R (2012): The symbiosis with arbuscular mycorrhizal fungi contributes to plant tolerance to serpentine edaphic stress. Soil Biology and Biochemistry 44, 56-64

Dudeja SS, Giri R, Saini R, Suneja-Madan P, Kothe E (2012): Interaction of endophytic microbes with legumes. Journal of Basic Microbiology 52, 248-260

Duffus JH (2002): "Heavy metals" a meaningless term? Pure and Applied Chemistry 74, 793-807

Duponnois R, Kisa M, Assigbetse K, Prin Y, Thioulouse J, Issartel M, Moulin P, Lepage M (2006): Fluorescent pseudomonads occuring in *Macrotermes subhyalinus* mound structures decrease Cd toxicity and improve its accumulation in sorghum plants. Science of The Total Environment 370, 391-400

Dushenkov S, Kapulnik Y, Blaylock M, Sorochisky B, Raskin I, Ensley B (1997): Phytoremediation: a novel approach to an old problem. In: Wise DL (Ed.), Studies in Environmental Science. Elsevier, 563-572

Dushenkov S, Vasudev D, Kapulnik Y, Gleba D, Fleisher D, Ting KC, Ensley B (1997): Removal of uranium from water using terrestrial plants. Environmental Science and Technology 31, 3468-3474

Dushenkov V, Kumar PB, Motto H, Raskin I (1995): Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environmental Science and Technology 29, 1239-45

Ebbs SD, Kochian LV (1998): Phytoextraction of zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*), and Indian mustard (*Brassica juncea*). Environmental Science and Technology 32, 802-806

Ebenå G, Kothe E (2006): Bioleaching and microbial mineral degradation. In: Varma A (Ed.), Microbes: Health and environment. Vol. 3, IK International Publishing, New Delhi, 389-405

El-Tarabily KA (2008): Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. Plant and Soil 308, 161-174

Epelde L, Mijangos I, Becerril JM, Garbisu C (2009): Soil microbial community as bioindicator of the recovery of soil functioning derived from metal phytoextraction with sorghum. Soil Biology and Biochemistry 41, 1788-1794

Erel Y, Torrent J (2010): Contribution of Saharan dust to Mediterranean soils assessed by sequential extraction and Pb and Sr isotopes. Chemical Geology 275, 19-25

Ernst WHO (2005): Phytoextraction of mine wastes - options and impossibilities. Chemie Der Erde-Geochemistry 65, 29-42

Escarré J, Lefébvre C, Gruber W, Leblanc M, Lepart J, Riviére Y, Delay B (2000): Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and

nonmetalliferous sites in the Mediterranean area: implications for phytoremediation. New Phytologist 145, 429-437

Evans LJ (1989): Chemistry of metal retention by soils. Environmental Science and Technology 23, 1046-1056

Farrag K, Senesi N, Nigro F, Petrozza A, Palma A, Shaarawi S, Brunetti G (2012): Growth responses of crop and weed species to heavy metals in pot and field experiments. Environmental Science and Pollution Research 19, 3636-3644

Fellet G, Marchiol L, Perosa D, Zerbi G (2007): The application of phytoremediation technology in a soil contaminated by pyrite cinders. Ecological Engineering 31, 207-214

Fernandez V, Winkelmann G (2005): The determination of ferric iron in plants by HPLC using the microbial iron chelator desferrioxamine E. BioMetals 18, 53-62

Fischerová Z, Tlustoš P, Jiřina S, Kornelie Š (2006): A comparison of phytoremediation capability of selected plant species for given trace elements. Environmental Pollution 144, 93-100

Franco-Correa M, Quintana A, Duque C, Suarez C, Rodriguez MX, Barea JM (2010): Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. Applied Soil Ecology 45, 209-217

Frankenberger WT, Arshad M (2001): Bioremediation of selenium-contaminated sediments and water. BioFactors 14, 241-254

Franzaring J (2014): Investigations on plant functional traits, epidermal structures and the ecophysiology of the novel bioenergy species *Sida hermaphrodita* Rusby and *Silphium perfoliatum* L. Journal of Applied Botany and Food Quality 87, 36-45

Gadd GM (2000): Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Current Opinion in Biotechnology 11, 271-279

Gadkari D, Morsdorf G, Meyer O (1992): Chemolithoautotrophic assimilation of dinitrogen by *Streptomyces thermoautotrophicus* UBT1: identification of an unusual N2-fixing system. Journal of Bacteriology 174, 6840-6843

Galli U, Schuepp H, Brunold C (1994): Heavy-metal binding by mycorrhizal fungi. Physiologia Plantarum 92, 364-368

Gansberger M, Montgomery LFR, Liebhard P (2015): Botanical characteristics, crop management and potential of *Silphium perfoliatum* L. as a renewable resource for biogas production: a review. Industrial Crops and Products 63, 362-372

Garbisu C, Alkorta I (2001): Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresource Technology 77, 229-236

Garcia G, Penas JM, Manteca JI (2008): Zn mobility and geochemistry in surface sulfide mining soils from SE Spain. Environmental Research 106, 333-339

Gardea-Torresdey JL, Peralta-Videa JR, de la Rosa G, Parsons JG (2005): Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. Coordination Chemistry Reviews 249, 1797-1810

Gary SB (1999): Factors influencing field phytoremediation of selenium-laden soils. In: Terry N (Ed.), Phytoremediation of contaminated soil and water. CRC Press

Gatzweiler R, Jakubick AT, Meyer J, Paul M, Schreyer J (2003): Flooding of the WISMUT mines – learning by doing or applying a comprehensive systematic approach? In: Merkel B, Planer-Friedrich B, Wolkersdorfer C (Eds.), Uranium in the aquatic environment. Springer, Heidelberg, 745-754

Gaur A, Adholeya A (2004): Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Current Science 86, 528-534

Geebelen W, Adriano DC, van der Lelie D, Mench M, Carleer R, Clijsters H, Vangronsveld J (2003): Selected bioavailability assays to test the efficacy of amendment-induced imobilization of lead in soils. Plant and Soil 249, 217-228

Germaine KJ, Liu X, Cabellos GG, Hogan JP, Ryan D, Dowling DN (2009): Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. FEMS Microbiology Ecology 57, 302-310

Germaine KJ, Keogh E, Ryan D, Dowling DN (2009): Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiology Letters 296, 226-234

Giller KE, Witter E, McGrath SP (1998): Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biology and Biochemistry 30, 1389-1414

Glick BR (2003): Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnology Advances 21, 383-393

Glick BR (2010): Using soil bacteria to facilitate phytoremediation. Biotechnology Advances 28, 367-374

Glick BR, Cheng Z, Czarny J, Duan J (2007): Promotion of plant growth by ACC deaminase-producing soil bacteria. European Journal of Plant Pathology 119, 329-339

Gold B, Deng H, Bryk R, Vargas D, Eliezer D, Roberts J, Jiang X, Nathan C (2008): Identification of a copper-binding metallothionein in pathogenic mycobacteria. Nature Chemical Biology 4, 609-616

Gonzalez-Guerrero M, Azcon-Aguilar C, Mooney M, Valderas A, MacDiarmid CW, Eide DJ, Ferrol N (2005): Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genetics and Biology 42, 130-140

Gonzalez-Guerrero M, Cano C, Azcon-Aguilar C, Ferrol N (2007): GintMT1 encodes a functional metallothionein in *Glomus intraradices* that responds to oxidative stress. Mycorrhiza 17, 327-335

Gopalakrishnan S, Pande S, Sharma M, Humayun P, Kiran BK, Sandeep D, Vidya MS, Deepthi K, Rupela O (2011): Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of *Fusarium* wilt of chickpea. Crop Protection 30, 1070-1078

Grawunder A, Lonschinski M, Merten D, Büchel G (2009): Distribution and bonding of residual contamination in glacial sediments at the former uranium mining leaching heap of Gessen/Thuringia, Germany. Chemie der Erde - Geochemistry 69, 5-19

Gray CW, McLaren RG, Roberts AHC (2003): Atmospheric accessions of heavy metals to some New Zealand pastoral soils. Science of The Total Environment 305, 105-115

Gray EJ, Smith DL (2005): Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biology and Biochemistry 37, 395-412

Griffioen WAJ (1994): Characterization of a heavy metal-tolerant endomycorrhizal fungus from the surroundings of a zinc refinery. Mycorrhiza 4, 197-200

Grill E, Löffler S, Winnacker EL, Zenk MH (1989): Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific γ-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). Proceedings of National Academy of Science USA 86, 6838-6842

Gtari M, Ghodhbane-Gtari F, Nouioui I, Beauchemin N, Tisa LS (2012): Phylogenetic perspectives of nitrogen-fixing actinobacteria. Archives of Microbiology 194, 3-11

Guo W, Zhao R, Zhao W, Fu R, Guo J, Bi N, Zhang J (2013): Effects of arbuscular mycorrhizal fungi on maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) grown in rare earth elements of mine tailings. Applied Soil Ecology 72, 85-92

Gupta N, Sahoo D, Basak UC (2010): Evaluation of in vitro solubilization potential of phosphate solubilising *Streptomyces* isolated from phyllosphere of *Heritiera fomes* (mangrove). African Journal of Microbiology Research 4, 136-142

Haferburg G, Kothe E (2007): Microbes and metals: interactions in the environment. Journal of Basic Microbiology 47, 453-467

Haferburg G, Merten D, Büchel G, Kothe E (2007a): Biosorption of metal and salt tolerant microbial isolates from a former uranium mining area. Their impact on changes in rare earth element patterns in acid mine drainage. Journal of Basic Microbiology 47, 474-484

Haferburg G, Reinicke M, Merten D, Büchel G, Kothe E (2007b): Microbes adapted to acid mine drainage as source for strains active in retention of aluminum or uranium. Journal of Geochemical Exploration 92, 196-204

Haferburg G, Klöß G, Schmitz W, Kothe E (2008): „Ni-struvite" - a new biomineral formed by a nickel resistant *Streptomyces acidiscabies*. Chemosphere 72, 517-523

Haferburg G, Kothe E (2010): Metallomics: lessons for metalliferous soil remediation. Applied Microbiology and Biotechnology 87, 1271-1280

Haferburg G, Kothe E (2012): Biogeosciences in heavy metal-contaminated soils. In: Kothe E, Varma A (Eds.), Bio-geo interactions in heavy metal-contaminated soils. Springer, Heidelberg, 17-34

Halim M, Conte P, Piccolo A (2003): Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. Chemosphere 52, 265-275

Hall JL (2002): Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany 53, 1-11

Hamdali H, Bouizgarne B, Hafidi M, Lebrihi A, Virolle MJ, Ouhdouch Y (2008a): Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines. Applied Soil Ecology 38, 12-19

Hamdali H, Hafidi M, Virolle M, Ouhdouch Y (2008b): Rock phosphate-solubilizing Actinomycetes: screening for plant growth-promoting activities. World Journal of Microbiology and Biotechnology 24, 2565-2575

Harrison MJ (2005): Signaling in the arbuscular mycorrhizal symbiosis. Annual Review of Microbiology 59, 19-42

Haselwandter K, Haninger G, Ganzera M (2011): Hydroxamate siderophores of the ectomycorrhizal fungi *Suillus granulatus* and *S. luteus*. BioMetals 24, 153-157

Haslmayr H-P, Meißner S, Langella F, Baumgarten A, Geletneky J (2014): Establishing best practice for microbially aided phytoremediation. Environmental Science and Pollution Research 21, 6765-6774

Hassan Z, Aarts MGM (2011): Opportunities and feasibilities for biotechnological improvement of Zn, Cd or Ni tolerance and accumulation in plants. Environmental and Experimental Botany 72, 53-63

Haynes RJ, Murtaza G, Naidu R (2009): Inorganic and organic constituents and contaminants of biosolids: implications for land application. In: Donald LS (Ed.), Advances in Agronomy. Academic Press, 165-267

Hernández-Allica J, Becerril JM, Garbisu C (2008): Assessment of the phytoextraction potential of high biomass crop plants. Environmental Pollution 152, 32-40

Hildebrandt A, Guillamón M, Lacorte S, Tauler R, Barceló D (2008): Impact of pesticides used in agriculture and vineyards to surface and groundwater quality (North Spain). Water Research 42, 3315-3326

Hildebrandt U, Janetta K, Bothe H (2002): Towards growth of arbuscular mycorrhizal fungi independent of a plant host. Applied and Environmental Microbiology 68, 1919-1924

Hildebrandt U, Regvar M, Bothe H (2007): Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68, 139-146

Hilson G, Murck B (2001): Progress toward pollution prevention and waste minimization in the North American gold mining industry. Journal of Cleaner Production 9, 405-415

Hindersmann I, Mansfeldt T (2014): Trace element solubility in a multimetal-contaminated soil as affected by redox conditions. Water, Air, and Soil Pollution 225, 1-20

Hiroki M (1992): Effects of heavy metal contamination on soil microbial population. Soil Science and Plant Nutrition 38, 141-147

Hodson ME (2004): Heavy metals--geochemical bogey men? Environmental Pollution 129, 341-343

Hohl H, Varma A (2010): Soil: the living matrix. In: Sherameti I, Varma A (Eds.), Soil Heavy Metals. Springer Berlin Heidelberg, 1-18

Hrynkiewicz K, Dabrowska G, Baum C, Niedojadlo K, Leinweber P (2012): Interactive and single effects of ectomycorrhiza formation and *Bacillus cereus* on metallothionein MT1 expression and phytoextraction of Cd and Zn by willows. Water, Air, and Soil Pollution 223, 957-968

Huang JWW, Chen JJ, Berti WR, Cunningham SD (1997): Phytoremediation of lead-contaminated soils: role of synthetic chelators in lead phytoextraction. Environmental Science and Technology 31, 800-805

Huang Y, Tao S, Chen YJ (2005): The role of arbuscular mycorrhiza on change of heavy metal speciation in rhizosphere of maize in wastewater irrigated agriculture soil. Journal of Environmental Science 17, 276-280

Jadia CD, Fulekar MH (2008): Phytotoxicity and remediation of heavy metals by fibrous root grass (sorghum). Journal of Applied Biosciences 10, 491-499

Jadia CD, Fulekar MH (2009): Phytoremediation of heavy metals: recent techniques. African Journal of Biotechnology 8, 921-928

Janouskova M, Pavlikova D, Vosatka M (2006): Potential contribution of arbuscular mycorrhiza to cadmium immobilisation in soil. Chemosphere 65, 1959-1965

Jarvis SC, Jones LHP (1980): The contents and sorption of cadmium in some agricultural soils of England and Wales. Journal of Soil Science 31, 469-479

Jasinskas A, Simonaviciute R, Siaudinis G, Liaudanskiene I, Antanaitis S, Arak M, Olt J (2014): The assessment of common mugwort (*Artemisia vulgaris* L.) and cup plant

(*Silphium perfoliatum* L.) productivity and technological preparation for solid biofuel. Agriculture 101, 19-26

Jentschke G, Godbold DL (2000): Metal toxicity and ectomycorrhizas. Physiologia Plantarum 109, 107-116

Ji GY, Silver S (1995): Bacterial-resistance mechanisms for heavy-metals of environmental concern. Journal of Industrial Microbiology 14, 61-75

Jing YD, He ZL, Yang XE (2007): Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. Journal of Zhejiang University Science B 8, 192-207

Jog R, Pandya M, Nareshkumar G, Rajkumar S (2014): Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiology 160, 778-788

Johnson DB, Hallberg KB (2005): Acid mine drainage remediation options: a review. Science of The Total Environment 338, 3-14

Joner E, Briones R, Leyval C (2000): Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant and Soil 226, 227-234

Jungwirth H, Kuchler K (2006): Yeast ABC transporters – a tale of sex, stress, drugs and aging. FEBS Letters 580, 1131-1138

Kagi JH, Kojima Y (1979): Nomenclature of metallothionein: a proposal. Experientia Supplementum 34, 141-142

Kaschl A, Romheld V, Chen Y (2002): Cadmium binding by fractions of dissolved organic matter and humic substances from municipal solid waste compost. Journal of Environmental Quality 31, 1885-1892

Kazakou E, Dimitrakopoulos PG, Baker AJM, Reeves RD, Troumbis AY (2008): Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level. Biological Reviews 83, 495-508

Keller C (2006): Factors limiting efficiency of phytoextraction at multi-metal contaminated sites. In: Morel J-L, Echevarria G, Goncharova N (Eds.), Phytoremediation of metal-contaminated soils. Springer Netherlands, 241-266

Khan AG (2005): Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. Journal of Trace Elements in Medicine and Biology 18, 355-364

Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000): Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41, 197-207

Kim FJ, Kim HP, Hah YC, Roe JH (1996): Differential expression of superoxide dismutases containing Ni and Fe/Zn in *Streptomyces coelicolor*. European Journal of Biochemistry 241, 178-185

Kim KR, Owens G, Naidu R, Kwon SI (2010): Influence of plant roots on rhizosphere soil solution composition of long-term contaminated soils. Geoderma 155, 86-92

Kim YJ, Song JY, Hong SK, Smith CP, Chang YK (2008): Effects of pH shock on the secretion system in *Streptomyces coelicolor* A3(2). Journal of Microbiology and Biotechnology 18, 658-662

Kloepper JW, Schroth MN (1979): Plant-growth promoting rhizobacteria – evidence that the mode of action involves root microflora interactions. Phytopathology 69, 1020-1024

Kloepper JW, Leong J, Teintze M, Schroth MN (1980): Enhanced plant-growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286, 885-886

Kloepper JW, Lifshitz R, Zablotowicz RM (1989): Free-living bacterial inocula for enhancing crop productivity. Trends in Biotechnology 7, 39-44

Kordel W, Dassenakis M, Lintelmann J, Padberg S (1997): The importance of natural organic material for environmental processes in waters and soils. Pure and Applied Chemistry 69, 1571-1600

Kothe E, Bergmann H, Büchel G (2005): Molecular mechanisms in bio-geo-interactions: from a case study to general mechanisms. Chemie der Erde - Geochemistry 65S1, 7-27

Kothe E, Dimkpa CO, Haferburg G, Schmidt A, Schmidt A, Schütze E (2010): Streptomycete heavy metal resistance: extracellular and intracellular mechanisms. In: Sherameti I, Varma A (Eds.), Soil Heavy Metals. Springer Berlin Heidelberg, 225-235

Kothe E, Dudeja SS (2011): Editorial. Journal of Basic Microbiology 51, 4

Krishna KR, Balakrishna AN, Bagyarajf DJ (1982): Interaction between a vesicular-arbuscular mycorrhizal fungus and *Streptomyces cinnamomeous* and their effects on finger millet. New Phytologist 92, 401-405

Kucharski R, Sas-Nowosielska A, Malkowski E, Japenga J, Kuperberg JM, Pogrzeba M, Krzyzak J (2005): The use of indigenous plant species and calcium phosphate for the stabilization of highly metal-polluted sites in southern Poland. Plant and Soil 273, 291-305

Kuffner M, Puschenreiter M, Wieshammer G, Gorfer M, Sessitsch A (2008): Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows. Plant and Soil 304, 35-44

Kuffner M, De Maria S, Puschenreiter M, Fallmann K, Wieshammer G, Gorfer M, Strauss J, Rivelli AR, Sessitsch A (2010): Culturable bacteria from Zn- and Cd-accumulating *Salix caprea* with differential effects on plant growth and heavy metal availability. Journal of Applied Microbiology 108, 1471-1484

Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995): Phytoextraction: the use of plants to remove heavy metals from soils. Environmental Science and Technology 29, 1232-1238

Kupper H, Zhao FJ, McGrath SP (1999): Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiology 119, 305-311

Kuyucak N, 2002. Role of microorganisms in mining: generation of acid rock drainage and its mitigation and treatment. European Journal of Mineral Processing and Environmental Protection 2, 179-196

Lagier T, Feuillade G, Matejka G (2000): Interactions between copper and organic macromolecules: determination of conditional complexation constants. Agronomie 20, 537-546

Lane B, Kajioka R, Kennedy T (1987): The wheat-germ Ec protein is a zinc-containing metallothionein. Biochemistry and Cell Biology 65, 1001-1005

Langella F, Grawunder A, Stark R, Weist A, Merten D, Haferburg G, Büchel G, Kothe E (2014): Microbially assisted phytoremediation approaches for two multi-element contaminated sites. Environmental Science and Pollution Research 21, 6845-6858

Lasat MM (2002): Phytoextraction of toxic metals: a review of biological mechanisms. Journal of Environmental Quality 31, 109-120

LeDuc DL, Terry N (2005): Phytoremediation of toxic trace elements in soil and water. Journal of Industrial Microbiology and Biotechnology 32, 514-520

Lee JH (2013): An overview of phytoremediation as a potentially promising technology for environmental pollution control. Biotechnology and Bioprocess Engineering 18, 431-439

Lehr NA, Schrey SD, Hampp R, Tarkka MT (2008): Root inoculation with a forest soil streptomycete leads to locally and systemically increased resistance against phytopathogens in Norway spruce. The New Phytologist 177, 965-976

Leyval C, Turnau K, Haselwandter K (1997): Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7, 139-153

Li WC, Ye ZH, Wong MH (2010): Metal mobilization and production of short-chain organic acids by rhizosphere bacteria associated with a Cd/Zn hyperaccumulating plant, *Sedum alfredii*. Plant and Soil 326, 453-467

Liang CC, Li T, Xiao YP, Liu MJ, Zhang HB, Zhao ZW (2009): Effects of inoculation with arbuscular mycorrhizal fungi on maize grown in multi-metal contaminated soils. International Journal of Phytoremediation 11, 692-703

Licht LA, Isebrands JG (2005): Linking phytoremediated pollutant removal to biomass economic opportunities. Biomass and Bioenergy 28, 203-218

Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M, van der Lelie D (2002): Endophytic bacteria and their potential applications. Critical Reviews in Plant Sciences 21, 583-606

Lonschinski M, Knöller K, Merten D, Büchel G (2011): Flow dynamics of groundwater and soil water in the former heap Gessenhalde at the uranium mining area of Ronneburg: a stable isotope approach. Hydrological Processes 25, 861-872

Ma Y, Rajkumar M, Freitas H (2009): Improvement of plant growth and nickel uptake by nickel resistant-plant-growth promoting bacteria. Journal of Hazardous Materials 166, 1154-1161

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011): Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnology Advances 29, 248-258

Macagnan D, Romeiro RD, Pomella AWV, deSouza JT (2008): Production of lytic enzymes and siderophores, and inhibition of germination of basidiospores of *Moniliophthora* (*ex Crinipellis*) *perniciosa* by phylloplane actinomycetes. Biological Control 47, 309-314

Macaskie LE (1991): The application of biotechnology to the treatment of wastes produced from the nuclear fuel cycle: Biodegradation and bioaccumulation as a means of treating radionuclide-containing streams. Critical Reviews in Biotechnology 11, 41-112

Maqsood MA, Hussain S, Aziz T, Ashraf M (2011): Wheat-exuded organic acids influence zinc release from calcareous soils. Pedosphere 21, 657-665

Marchiol L, Fellet G, Perosa D, Zerbi G (2007): Removal of trace metals by *Sorghum bicolor* and *Helianthus annuus* in a site polluted by industrial wastes: a field experience. Plant Physiology and Biochemistry 45, 379-387

Marques APGC, Moreira H, Franco AR, Rangel AOSS, Castro PML (2013): Inoculating *Helianthus annuus* (sunflower) grown in zinc and cadmium contaminated soils with plant growth promoting bacteria – effects on phytoremediation strategies. Chemosphere 92, 74-83

Masarovicova E, Kralova K, Pesko M (2009): Energetic Plants - cost and benefit. Ecological Chemistry and Engineering S 16, 263-276

Mastretta C, Barac T, Vangronsveld J, Newman L, Taghavi S, van der Lelie D (2006): Endophytic bacteria and their potential application to improve the phytoremediation of contaminated environments. Biotechnology and Genetic Engineering Reviews 23, 175-207

McGrath SP, Sidoli CMD, Baker AJM, Reeves RD (1993): The potential for the use of metal-accumulating plants for the in situ decontamination of metal-polluted soils. In: Eijsackers HJP, Hamers T (Eds.), Integrated soil and sediment research: a basis for proper prediction. Kluwer, Dordrecht, The Netherlands, 673-676

McGrath SP, Zhao FJ (2003): Phytoextraction of metals and metalloids from contaminated soils. Current Opinion in Biotechnology 14, 277-282

McGrath SP, Zhao FJ, Lombi E (2001): Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant and Soil 232, 207-214

McIntyre T (2003): Phytoremediation of heavy metals from soils. In: Tsao D (Ed.), Phytoremediation. Springer Berlin Heidelberg, 97-123

Meers E, Ruttens A, Hopgood M, Lesage E, Tack FMG (2005): Potential of *Brassic rapa*, *Cannabis sativa*, *Helianthus annuus* and *Zea mays* for phytoextraction of heavy metals from calcareous dredged sediment derived soils. Chemosphere 61, 561-572

Memon AR, Schröder P (2009): Implications of metal accumulation mechanisms to phytoremediation. Environmental Science and Pollution Research 16, 162-175

Mendez MO, Maier RM (2008): Phytostabilization of mine tailings in arid and semiarid environments - an emerging remediation technology. Environmental Health Perspectives 116, 278-283

Merten D, Geletneky J, Bergmann H, Haferburg G, Kothe E, Büchel G (2005): Rare earth element patterns: a tool for remediation of acid mine drainage. Chemie der Erde - Geochemistry 65S1, 97-114

Mirgorodsky D, Ollivier D, Merten D, Bergmann H, Büchel G, Willscher S, Wittig J, Jablonski L, Werner P (2010): Radiation protection of radioactively contaminated large areas by phytoremediation and subsequent utilization of the contaminated plant residues. Journal for Nuclear Power 55, 774-778

Misaghi IJ, Donndelinger CR (1990): Endophytic bacteria in symptom-free cotton plants. Phytopathology 80, 808-811

Moreira MAS, Souza ECd, Moraes CAd (2004): Multidrug efflux systems in gram-negative bacteria. Brazilian Journal of Microbiology 35, 19-28

Moreira H, Marques AGC, Franco A, Rangel ASS, Castro PL (2014): Phytomanagement of Cd-contaminated soils using maize (*Zea mays* L.) assisted by plant growth-promoting rhizobacteria. Environmental Science and Pollution Research 21, 9742-9753

Mulligan CN, Yong RN, Gibbs BF (1999): On the use of biosurfactants for the removal of heavy metals from oil-contaminated soil. Environmental Progress 18, 50-54

Mulligan CN, Yong RN, Gibbs BF (2001): Heavy metal removal from sediments by biosurfactants. Journal of Hazardous Materials 85, 111-125

Mulligan CN, Yong RN, Gibbs BF (2001): Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Engineering Geology 60, 193-207

Murakami M, Ae N (2009): Potential for phytoextraction of copper, lead, and zinc by rice (*Oryza sativa* L.), soybean (*Glycine max* [L.] Merr.), and maize (*Zea mays* L.). Journal of Hazardous Materials 162, 1185-1192

Murillo JM, Maranon T, Cabrera F, Lopez R (1999): Accumulation of heavy metals in sunflower and sorghum plants affected by the Guadiamar spill. Science of the Total Environment 242, 281-292

Murphy A, Zhou JM, Goldsbrough PB, Taiz L (1997): Purification and immunological identification of metallothioneins 1 and 2 from *Arabidopsis thaliana*. Plant Physiology 113, 1293-1301

Nagajyoti PC, Lee KD, Sreekanth TVM (2010): Heavy metals, occurrence and toxicity for plants: a review. Environmental Chemistry Letters 8, 199-216

Nagpure A, Choudhary B, Kumar S, Gupta RK (2014): Isolation and characterization of chitinolytic *Streptomyces* sp MT7 and its antagonism towards wood-rotting fungi. Annals of Microbiology 64, 531-541

Naidu R, Kookana RS, Sumner ME, Harter RD, Tiller KG (1997): Cadmium sorption and transport in variable charge soils: a review. Journal of Environmental Quality 26, 602-617

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G, Valori F (2008): Effects of root exudates in microbial diversity and activity in rhizosphere soils. In: Nautiyal C, Dion P (Eds.), Molecular Mechanisms of Plant and Microbe Coexistence. Springer Berlin Heidelberg, 339-365

Narula N, Reinicke M, Haferburg G, Kothe E, Behl RK (2012): Plant-microbe interaction in heavy metal-contaminated soils. In: Kothe E, Varma A (Eds.), Bio-geo interactions in heavy metal-contaminated soils. Springer, Heidelberg, 143-162

Neagoe A, Ebena G, Carlsson E (2005): The effect of soil amendments on plant performance in an area affected by acid mine drainage. Chemie Der Erde-Geochemistry 65, 115-129

Neagoe A, Stancu P, Nicoara A, Onete M, Bodescu F, Gheorghe R, Iordache V (2014): Effects of arbuscular mycorrhizal fungi on *Agrostis capillaris* grown on amended mine tailing substrate at pot, lysimeter, and field plot scales. Environmental Science and Pollution Research 21, 6859-6876

Neilands JB (1984): Methodology of siderophores. Structure and Bonding 58, 1-24

Newman LA, Reynolds CM (2005): Bacteria and phytoremediation: new uses for endophytic bacteria in plants. Trends in Biotechnology 23, 6-8

Nicholson FA, Chambers BJ, Williams JR, Unwin RJ (1999): Heavy metal contents of livestock feeds and animal manures in England and Wales. Bioresource Technology 70, 23-31

Nicholson FA, Smith SR, Alloway BJ, Carlton-Smith C, Chambers BJ (2003): An inventory of heavy metals inputs to agricultural soils in England and Wales. The Science of The Total Environment 311, 205-219

Nicholson FA, Rollett, A, Chambers, BJ (2010): The Defra “Agricultural soil heavy metal inventory for 2008” (Report 3 for Defra Project SP0569), Nottinghamshire, ADAS

Nicks L, Chambers MF (1998): A pioneering study of the potential of phytomining for nickel. In: Brooks RR (Ed.), Plants that hyperaccumulate heavy metals. CAB International, Wallingford, 312-325

Nicks L, Chambers MF (1995): Farming for metals. Mining Environment Meeting 199, 15-18

Nicoara A, Neagoe A, Stancu P, de Giudici G, Langella F, Sprocati AR, Iordache V, Kothe E (2014): Coupled pot and lysimeter experiments assessing plant performance in microbially assisted phytoremediation. Environmental Science and Pollution Research 21, 6905-6920

Nies DH (1992): Resistance to cadmium, cobalt, zinc, and nickel in microbes. Plasmid 27, 17-28

Nies DH, Silver S (1995): Ion efflux systems involved in bacterial metal resistances. Journal of Industrial Microbiology 14, 186-199

Nies DH (1999): Microbial heavy-metal resistance. Applied Microbiology and Biotechnology 51, 730-750

Nies DH (2003): Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiology Reviews 27, 313-339

Nigam R, Srivastava S, Prakash S, Srivastava MM (2001): Cadmium mobilisation and plant availability - the impact of organic acids commonly exuded from roots. Plant and Soil 230, 107-113

Nikaido H (1996): Multidrug efflux pumps of gram-negative bacteria. Journal of Bacteriology 178, 5853-5859

Nogueira MA, Nehls U, Hampp R, Poralla K, Cardoso E (2007): Mycorrhiza and soil bacteria influence extractable iron and manganese in soil and uptake by soybean. Plant and Soil 298, 273-284

Norvell WA (1984): Comparison of chelating agents as extractants for metals in diverse soil materials. Soil Science Society of America Journal 48, 1285-1292

Nowack B, Koehler S, Schulin R (2004): Use of diffusive gradients in thin films (DGT) in undisturbed field soils. Environmental Science and Technology 38, 1133-1138

Nriagu JO (1988): A silent epidemic of environmental metal poisoning? Environmental pollution 50, 139-161

Orłowska E, Zubek S, Jurkiewicz A, Szarek- Łukaszewska G, Turnau K (2002): Influence of restoration on arbuscular mycorrhiza of *Biscutella laevigata* L. (Brassicaceae) and *Plantago lanceolata* L. (Plantaginaceae) from calamine spoil mounds. Mycorrhiza 12, 153-159

Padmavathiamma PK, Li LY (2007): Phytoremediation Technology: Hyper-accumulation metals in plants. Water, Air, and Soil Pollution 184, 105-126

Palaniyandi SA, Yang SH, Suh JW (2013): Extracellular proteases from *Streptomyces phaeopurpureus* ExPro138 inhibit spore adhesion, germination and appressorium formation in *Colletotrichum coccodes*. Journal of Applied Microbiology 115, 207-217

Pankratov TA, Dedysh SN (2009): Cellulolytic streptomycetes from *Sphagnum* peat bogs and factors controlling their activity. Microbiology 78, 227-233

Park JH, Lamb D, Paneerselvam P, Choppala G, Bolan N, Chung J-W (2011): Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. Journal of Hazardous Materials 185, 549-574

Peppas A, Komnitsas K, Halikia I (2000): Use of organic covers for acid mine drainage control. Minerals Engineering 13, 563-574

Phipps DA (1981): Chemistry and biochemistry of trace metals in biological systems. In: Lepp NW (Ed.), Effect of Heavy Metal Pollution on Plants. Springer Netherlands, 1-54

Pichtel JR, Dick WA, Sutton P (1994): Comparison of amendments and management practices for long-term reclamation of abandoned mine lands. Journal of Environmental Quality 23, 766-772

Pilon-Smits E (2005): Phytoremediation. Annual Review of Plant Biology 56, 15-39

Pitman M (1972): Uptake and transport of ions in barley seedlings II. Evidence for two active stages in transport to the shoot. Australian Journal of Biological Science 25, 243-258

Pocsi I, Prade RA, Penninckx MJ (2004): Glutathione, altruistic metabolite in fungi. Advances in Microbial Physiology 49, 1-76

Polti M, Amoroso MJ, Abate CM (2011): Intracellular chromium accumulation by *Streptomyces* sp. MC1. Water, Air, and Soil Pollution 214, 49-57

Postolaky O, Baltsat K, Burtseva S, Maslobrod S (2012): Effect of *Streptomyces* metabolites on some physiological parameters of maize seeds. Agriculture 69, 23-29

Postolaky O, Burtseva S, Baltsat C, Barbac T, Chiselitsa O, Maslobrod S (2013): Effect of *Streptomyces* metabolites on some physiological parameters of triticale seeds. Agronomy 56, 500-504

Prasad MNV (2004): Phytoremediation of metals and radionuclides in the environment: the case for natural hyperaccumulators, metal transporters, soil-amending chelators and transgenic plants. In: Prasad MNV (Ed.), Heavy metal stress in plants. Springer Berlin Heidelberg, 345-391

Prasad MNV (2005): Nickelophilous plants and their significance in phytotechnologies. Brazilian Journal of Plant Physiology 17, 113-128

Pratas J, Prasad MNV, Freitas H, Conde L (2005): Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. Journal of Geochemical Exploration 85, 99-107

Pulford ID, Watson C (2003): Phytoremediation of heavy metal-contaminated land by trees - a review. Environment International 29, 529-540

Pyle DM, Mather TA (2003): The importance of volcanic emissions for the global atmospheric mercury cycle. Atmospheric Environment 37, 5115-5124

Quartacci MF, Irtelli B, Gonnelli C, Gabbrielli R, Navari-Izzo F (2009): Naturally-assisted metal phytoextraction by *Brassica carinata*: role of root exudates. Environmental Pollution 157, 2697-2703

Rajapaksha RMCP, Tobor-Kapłon MA, Bååth E (2004): Metal toxicity affects fungal and bacterial activities in soil differently. Applied and Environmental Microbiology 70, 2966-2973

Rajkumar M, Ae N, Freitas H (2009): Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77, 153-160

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010): Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends in Biotechnology 28, 142-149

Raman N, Nagarajan N, Gopinathan S, Sambandan K (1993): Mycorrhizal status of plant species colonizing a magnesite mine spoil in India. Biology and Fertility of Soils 16, 76-78

Ramos L, Hernandez LM, Gonzalez MJ (1994): Sequential fractionation of copper, lead, cadmium and zinc in soils from or near Doñana National Park. Journal of Environmental Quality 23, 50-57

Rascio N, Navari-Izzo F (2011): Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Science 180, 169-181

Raskin I, Kumar PBAN, Dushenkov S, Salt DE (1994): Bioconcentration of heavy metals by plants. Current Opinion in Biotechnology 5, 285-290

Raskin I, Smith RD, Salt DE (1997): Phytoremediation of metals: using plants to remove pollutants from the environment. Current Opinion in Biotechnology 8, 221-226

Raskin I, Salt DE, Krämer U, Schulman R (1998): Phytoremediation: green and clean. Acta Horticulturae 457, 329-332

Rauser WE (1995): Phytochelatins and related peptides (structure, biosynthesis, and function). Plant Physiology 109, 1141-1149

Reinicke M, Schindler F, Roth M, Kothe E (2013): Multi-metal bioremediation by microbial assisted phytoremediation. In: Amoroso MJ, Benimeli CS, Cuozzo SA (Eds.), Actinobacteria: Application in bioremediation and production of industrial enzymes. CRC Press, Boca Raton, USA, 87-105

Rho JY, Kim JH (2002): Heavy metal biosorption and its significance to metal tolerance of streptomycetes. Journal of Microbiology 40, 51-54

Ribbe M, Gadkari D, Meyer O (1997): N_2 fixation by *Streptomyces thermoautotrophicus* involves a molybdenum-dinitrogenase and a manganese-superoxide oxidoreductase that couple N_2 reduction to the oxidation of superoxide produced from O_2 by a molybdenum-CO dehydrogenase. Journal of Biological Chemistry 272, 26627-26633

Riedlinger J, Schrey SD, Tarkka MT, Hampp R, Kapur M, Fiedler HP (2006): Auxofuran, a novel metabolite that stimulates the growth of fly agaric, is produced by the mycorrhiza helper bacterium *Streptomyces* strain AcH 505. Applied and Environmental Microbiology 72, 3550-3557

Rieuwerts JS, Thornton I, Farago ME, Ashmore MR (1998): Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. Chemical Speciation and Bioavailability 10, 61-75

Riley PA (1997): Melanin. The International Journal of Biochemistry and Cell Biology 29, 1235-1239

Rinaldi AC, Comandini O, Kuyper TW (2008): Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Diversity 33, 1-45

Robinson BH, Brooks RR, Howes AW, Kirkman JH, Gregg PEH (1997): The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. Journal of Geochemical Exploration 60, 115-126

Robinson BH, Mills TM, Petit D, Fung LE, Green SR, Clothier BE (2000): Natural and induced cadmium-accumulation in poplar and willow: implications for phytoremediation. Plant and Soil 227, 301-306

Robinson BH, Banuelos G, Conesa HM, Evangelou MWH, Schulin R (2009): The phytomanagement of trace elements in soil. Critical Reviews in Plant Sciences 28, 240-266

Rojas-Tapias D, Bonilla R, Dussán J (2012): Effect of inoculation with plant growth-promoting bacteria on growth and copper uptake by sunflowers. Water, Air, and Soil Pollution 223, 643-654

Ross SM (1994): Toxic metals in soil-plant systems. John Wiley and Sons, Chichester

Rózycki H, Strzelczyk E (1986): Organic acids production by *Streptomyces* spp. isolated from soil, rhizosphere and mycorrhizosphere of pine (*Pinus sylvestris* L.). Plant and Soil 96, 337-345

Ryan P, Delhaize E, Jones D (2001): Function and mechanism of organic anion exudation from plant roots. Annual Review of Plant Physiology and Plant Molecular Biology 52, 527-560

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008): Bacterial endophytes: recent developments and applications. FEMS Microbiology Letters 278, 1-9

Salt DE, Blaylock M, Kumar N, Dushenkov V, Ensley BD, Chet I, Raskin I (1995): Phytoremediation - a novel strategy for the removal of toxic metals from the environment using plants. Bio-Technology 13, 468-474

Salt DE, Smith RD, Raskin I (1998): Phytoremediation. Annual Review of Plant Physiology and Plant Molecular Biology 49, 643-668

Sánchez-Martín MJ, García-Delgado M, Lorenzo LF, Rodríguez-Cruz MS, Arienzo M (2007): Heavy metals in sewage sludge amended soils determined by sequential extractions as a function of incubation time of soils. Geoderma 142, 262-273

Saraswat S, Rai JPN (2009): Phytoextraction potential of six plant species grown in multimetal contaminated soil. Chemistry and Ecology 25, 1-11

Schalk IJ, Hannauer M, Braud A (2011): New roles for bacterial siderophores in metal transport and tolerance. Environmental Microbiology 13, 2844-2854

Schindler F, Gube M, Kothe E (2012): Bioremediation and heavy metal uptake: Microbial approaches at field scale. In: Kothe E, Varma A (Eds.), Bio-geo interactions in metal-contaminated soils. Springer Berlin Heidelberg, 365-383

Schmidt A, Haferburg G, Sineriz M, Merten D, Büchel G, Kothe E (2005): Heavy metal resistance mechanisms in actinobacteria for survival in AMD contaminated soils. Chemie Der Erde-Geochemistry 65, 131-144

Schmidt A, Schmidt A, Haferburg G, Kothe E (2007): Superoxide dismutases of heavy metal resistant streptomycetes. Journal of Basic Microbiology 47, 56-62

Schmidt A, Haferburg G, Schmidt A, Lischke U, Merten D, Ghergel F, Büchel G, Kothe E (2009): Heavy metal resistance to the extreme: *Streptomyces* strains from a former uranium mining area. Chemie Der Erde-Geochemistry 69, 35-44

Schmidt A, Hagen M, Schutze E, Schmidt A, Kothe E (2010): *In silico* prediction of potential metallothioneins and metallohistins in actinobacteria. Journal of Basic Microbiology 50, 562-569

Schreiner RP, Koide RT (1993): Streptomycin reduces plant response to mycorrhizal infection. Soil Biology and Biochemistry 25, 1131-1133

Schrempf H (2001): Recognition and degradation of chitin by streptomycetes. Antonie Van Leeuwenhoek 79, 285-289

Schrempf H, Koebsch I, Walter S, Engelhardt H, Meschke H (2011): Extracellular *Streptomyces* vesicles: amphorae for survival and defence. Microbial Biotechnology 4, 286-299

Schrey SD, Erkenbrack E, Fruh E, Fengler S, Hommel K, Horlacher N, Schulz D, Ecke M, Kulik A, Fiedler HP, Hampp R, Tarkka MT (2012): Production of fungal and bacterial growth modulating secondary metabolites is widespread among mycorrhiza-associated streptomycetes. BMC Microbiology 12:164

Schultz PA, Miller RM, Jastrow JD, Rivetta CV, Bever JD (2001): Evidence of a mycorrhizal mechanism for the adaptation of *Andropogon gerardii* (Poaceae) to high- and low-nutrient prairies. American Journal of Botany 88, 1650-1656

Schütze E, Kothe E (2012): Heavy metal-resistant streptomycetes in soil. In: Kothe E , Varma A (Eds.), Bio-geo interactions in metal-contaminated soils. Springer Berlin Heidelberg, 163-182

Schütze E, Klose M, Merten D, Nietzsche S, Senftleben D, Roth M, Kothe E (2014): Growth of streptomycetes in soil and their impact on bioremediation. Journal of Hazardous Materials 267, 128-135

Schuwirth N, Hofmann T (2006): Comparability of and alternatives to leaching tests for the assessment of the emission of inorganic soil contamination. Journal of Soils and Sediments 6, 102-112

Selosse MA, Baudoin E, Vandenkoornhuyse P (2004): Symbiotic microorganisms, a key for ecological success and protection of plants. Critical Reviews in Biology 327, 639-648

Senitz S, Gaupp R, Büchel G (2009): Hochaufgelöste dreidimensionale Rekonstruktion eines quartären Talaquifers in einem Teilbereich des Gessentals bei Ronneburg (ehemaliges ostthüringisches Uranerzbergbaurevier) durch die Kombination von Geländedaten und geostatistischer Schätzung. Zeitschrift der deutschen Gesellschaft für Geowissenschaften 160, 367-389

Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K, Puschenreiter M (2013): The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biology and Biochemistry 60, 182-194

Sheng X, Sun L, Huang Z, He L, Zhang W, Chen Z (2012): Promotion of growth and Cu accumulation of bio-energy crop (*Zea mays*) by bacteria: implications for energy plant biomass production and phytoremediation. Journal of Environmental Management 103, 58-64

Sheoran V, Sheoran AS, Poonia P (2011): Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review. Critical Reviews in Environmental Science and Technology 41, 168-214

Shetty KG, Hetrick BAD, Figge DAH, Schwab AP (1994): Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. Environmental Pollution 86, 181-188

Shetty KG, Hetrick BAD, Schwab AP (1995): Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. Environmental Pollution 88, 307-314

Shi JY, Lin HR, Yuan XF, Chen XC, Shen CF, Chen YX (2011): Enhancement of copper availability and microbial community changes in rice rhizospheres affected by sulfur. Molecules 16, 1409-1417

Siaudinis G, Jasinskas A, Slepetiene A, Karcauskiene D (2012): The evaluation of biomass and energy productivity of common mugwort (*Artemisia vulgaris* L.) and cup plant (*Silphium perfoliatum* L.) in Albeluvisol. Agriculture 99, 357-362

Silver S, Phung LT (1996): Bacterial heavy metal resistance: new surprises. Annual Review of Microbiology 50, 753-789

Sineriz ML, Kothe E, Abate CM (2009): Cadmium biosorption by *Streptomyces* sp. F4 isolated from former uranium mine. Journal of Basic Microbiology 49, 55-S62

Singh JS, Pandey VC, Singh DP (2011): Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agriculture, Ecosystems and Environment 140, 339-353

Smith SE, Read DJ (1997): Mycorrhizal symbiosis. Academic Press, San Diego

Smith SE, Smith FA (2011): Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annual Review of Plant Biology 62, 227-250

Sobolev D, Begonia MF (2008): Effects of heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. International Journal of Environmental Research and Public Health 5, 450-456

Sousa CD, Soares ACF, Garrido MD (2008): Characterization of streptomycetes with potential to promote plant growth and biocontrol. Scientia Agricola 65, 50-55

Sprocati AR, Alisi C, Pinto V, Montereali MR, Marconi P, Tasso F, Turnau K, De Giudici G, Goralska K, Bevilacqua M, Marini F, Cremisini C (2014): Assessment of the applicability of a "toolbox" designed for microbially assisted phytoremediation: the case study at Ingurtosu mining site (Italy). Environmental Science and Pollution Research 21, 6939-6951

Stephan UW, Schmidke I, Stephan VW, Scholz G (1996): The nicotianamine molecule is made-to-measure for complexation of metal micronutrients in plants. BioMetals 9, 84-90

Stern BR (2010): Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations. Journal of Toxicology and Environmental Health 73, 114-127

Ström L, Owen AG, Godbold DL, Jones DL (2005): Organic acid behaviour in a calcareous soil implications for rhizosphere nutrient cycling. Soil Biology and Biochemistry 37, 2046-2054

Sullivan TS, McBride MB, Thies JE (2013): Rhizosphere microbial community and Zn uptake by willow (*Salix purpurea* L.) depend on soil sulfur concentrations in metalliferous peat soils. Applied Soil Ecology 67, 53-60

Tak H, Ahmad F, Babalola OO (2013): Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. In: Whitacre DM (Ed.), Reviews of environmental contamination and toxicology Volume 223. Springer New York, 33-52

Tanimoto E (2005): Regulation of root growth by plant hormones - roles for auxin and gibberellin. Critical Reviews in Plant Sciences 24, 249-265

Tao S, Liu WX, Chen YJ, Xu FL, Dawson RW, Li BG, Cao J, Wang XJ, Hu JY, Fang JY (2004): Evaluation of factors influencing root-induced changes of copper fractionation in rhizosphere of a calcareous soil. Environmental Pollution 129, 5-12

Tapio E, Pohto-Lahdenperä A (1991): Scanning electron microscopy of hyphal interaction between *Streptomyces griseoviridis* and some plant pathogenic fungi. Journal of Agricultural Science in Finland 63, 435-441

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012): Heavy metals toxicity and the environment. Experientia Supplementum 101, 133-164

Teale WD, Paponov IA, Palme K (2006): Auxin in action: signalling, transport and the control of plant growth and development. Nature Reviews Molecular Cell Biology 7, 847-859

Thumann J, Grill E, Winnacker EL, Zenk MH (1991): Reactivation of metal-requiring apoenzymes by phytochelatin-metal complexes. FEBS Letters 284, 66-69

Tlustos P, Szakova J, Hruby J, Hartman I, Najmanova J, Nedelnik J, Pavlikova D, Batysta M (2006): Removal of As, Cd, Pb, and Zn from contaminated soil by high biomass producing plants. Plant, Soil and Environment 52, 413-423

Toler HD, Morton JB, Cumming JR (2005): Growth and metal accumulation of mycorrhizal sorghum exposed to elevated copper and zinc. Water, Air, and Soil Pollution 164, 155-172

Tonin C, Vandenkoornhuyse P, Joner EJ, Straczek J, Leyval C (2001): Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. Mycorrhiza 10, 161-168

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI (2006): Microbial producers of plant growth stimulators and their practical use: a review. Applied Biochemistry and Microbiology 42, 117-126

Turnau K, Kottke I, Dexheimer J, Botton B (1994): Element distribution in mycelium of *Pisolithus arrhizus* treated with cadmium dust. Annals of Botany 74, 137-142

Turnau K, Ryszka P, Gianinazzi-Pearson V, van Tuinen D (2001): Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. Mycorrhiza 10, 169-174

Turnau K, Anielska T, Ryszka P, Gawroński S, Ostachowicz B, Jurkiewicz A (2008): Establishment of arbuscular mycorrhizal plants originating from xerothermic grasslands on heavy metal rich industrial wastes - new solution for waste revegetation. Plant and Soil 305, 267-280

Unterbrunner R, Puschenreiter M, Sommer P, Wieshammer G, Tlustos P, Zupan M, Wenzel WW (2007): Heavy metal accumulation in trees growing on contaminated sites in Central Europe. Environmental Pollution 148, 107-114

Usman ARA, Mohamed HM (2009): Effect of microbial inoculation and EDTA on the uptake and translocation of heavy metal by corn and sunflower. Chemosphere 76, 893-899

Vamerali T, Bandiera M, Mosca G (2009): Field crops for phytoremediation of metal-contaminated land. a review. Environmental Chemistry Letters 8, 1-17

Van Nevel L, Mertens J, Oorts K, Verheyen K (2007): Phytoextraction of metals from soils: how far from practice? Environmental Pollution 150, 34-40

Vangronsveld J, Colpaert JV, van Tichelen KK (1996): Reclamation of a bare industrial area contaminated by non-ferrous metals: physico-chemical and biological evaluation of the durability of soil treatment and revegetation. Environmental Pollution 94, 131-140

Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, van der Lelie D, Mench M (2009): Phytoremediation of contaminated soils and groundwater: lessons from the field. Environmental Science and Pollution Research International 16, 765-794

Varekamp JC, Buseck PR (1986): Global mercury flux from volcanic and geothermal sources. Applied Geochemistry 1, 65-73

Vetter A, Conrad M, Biertümpfel A (2007): Energiepflanzen für die Biogasproduktion – Teilvorhaben 2: Optimierung der Verfahrenskette der Bereitstellung und Nutzung

von Energiepflanzen zur Kofermentation im Biogasreaktor (Abschlussbericht No. 42.22.430), Thüringer Landesanstalt für Landwirtschaft, Jena

Violante A, Cozzolino V, Perelomov L, Caporale AG, Pigna M (2010): Mobility and bioavailability of heavy metals and metalloids in soil environments. Journal of Soil Science and Plant Nutrition 10, 268-292

Vögeli-Lange R, Wagner GJ (1990): Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves: implication of a transport function for cadmium-binding peptides. Plant Physiology 92, 1086-1093

Voigt TB, Lee DK, Kling GJ (2012): Perennial herbaceous crops with potential for biofuel production in the temperate regions of the USA. CAB Reviews 7, 1-13

Waksman SA, Reilly HC, Johnstone DB (1946): Isolation of streptomycin-producing strains of *Streptomyces griseus*. Journal of Bacteriology 52, 393-397

Wang B, Qiu YL (2006): Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16, 299-363

Wang FY, Lin XG, Yin R (2007): Inoculation with arbuscular mycorrhizal fungus *Acaulospora mellea* decreases Cu phytoextraction by maize from Cu-contaminated soil. Pedobiologia 51, 99-109

Welch RM, Norwell WA, Schäfer SC, Shaff JE, Kochian LV (1993): Induction of iron(III) and copper(II) reduction in pea (*Pisum sativum* L.) roots by Fe and Cu status – does the root-cell plasmalemma Fe(III)-chelate reductase perform a general role in regulating cation uptake. Planta 190, 555-561

Wernitznig S, Adlassnig W, Sprocati AR, Turnau K, Neagoe A, Alisi C, Sassmann S, Nicoara A, Pinto V, Cremisini C, Lichtscheidl I (2014): Plant growth promotion by inoculation with selected bacterial strains versus mineral soil supplements. Environmental Science and Pollution Research 21, 6877-6887

Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J (2009): Exploiting plant–microbe partnerships to improve biomass production and remediation. Trends in Biotechnology 27, 591-598

Weyens N, Croess Dupae J, Newman L, van der Lelie D, Carleer R, Vangronsveld J (2010): Endophytic bacteria improve phytoremediation of Ni and TCE co-contamination. Environmental Pollution 158, 2422-2427

Wilkins DA (1991): The influence of sheathing (ecto-) mycorrhizas of trees on the uptake and toxicity of metals. Agriculture, Ecosystems and Environment 35, 245-260

Willscher S, Mirgorodsky D, Jablonski L, Ollivier D, Merten D, Büchel G, Wittig J, Werner P (2013): Field scale phytoremediation experiments on a heavy metal and uranium contaminated site, and further utilization of the plant residues. Hydrometallurgy 131-132, 46-53

Yanez L, Ortiz D, Calderon J, Batres L, Mejia J, Martinez L, Garcia-Nieto E, Diaz-Barriga F (2002): Overview of human health and chemical mixtures: problems facing developing countries. Environmental Health Perspectives 110, 901-909

Yang SS, Wang JY (1999): Protease and amylase production of *Streptomyces rimosus* in submerged and solid state cultivations. Botanical Bulletin of Academia Sinica 40, 259-265

Yang XE, Feng Y, He Z, Stoffella PJ (2005): Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. Journal of Trace Elements in Medicine and Biology 18, 339-353

Yang XE, Jin XF, Feng Y, Islam E (2005): Molecular mechanisms and genetic basis of heavy metal tolerance/hyperaccumulation in plants. Journal of Integrative Plant Biology 47, 1025-1035

Yoon J, Cao XD, Zhou QX, Ma LQ (2006): Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Science of the Total Environment 368, 456-464

Zaidi A, Khan MS, Ahemad M, Oves M (2009): Plant growth promotion by phosphate solubilizing bacteria. Acta microbiologica et immunologica Hungarica 56, 263-284

Zeien H, Brümmer G (1989): Chemische Extraktionen zur Bestimmung von Schwermetallbindungsformen in Böden. Mitteilungen der Deutschen Bodenkundlichen Gesellschaft 59, 505-510

Zhang X, Xia H, Li Z, Zhuang P, Gao B (2010): Potential of four forage grasses in remediation of Cd and Zn contaminated soils. Bioresource Technology 101, 2063-2066

Zhao FJ, Lombi E, McGrath SP (2003): Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant and Soil 249, 37-43

Zhao S, Du CM, Tian CY (2012): Suppression of *Fusarium oxysporum* and induced resistance of plants involved in the biocontrol of Cucumber Fusarium Wilt by *Streptomyces bikiniensis* HD-087. World Journal of Microbiology and Biotechnology 28, 2919-2927

Zhou JM, Dang Z, Chen NC, Xu SG, Xie ZY (2007): Enhanced phytoextraction of heavy metal contaminated soil by chelating agents and auxin indole-3-acetic acid. Huang Jing Ke Xue 28, 2085-2088

Zhou X, He Z, Liang Z, Stoffella PJ, Fan J, Yang Y, Powell CA (2011): Long-term use of copper-containing fungicide affects microbial properties of citrus grove soils. Soil Science Society of America Journal 75, 898-906

Zhuang P, Yang QW, Wang HB, Shu WS (2007): Phytoextraction of heavy metals by eight plant species in the field. Water, Air, and Soil Pollution 184, 235-242

Zhuang P, Shu W, Li Z, Liao B, Li J, Shao J (2009): Removal of metals by sorghum plants from contaminated land. Journal of Environmental Sciences 21, 1432-1437

Zhuang X, Chen J, Shim H, Bai Z (2007): New advances in plant growth-promoting rhizobacteria for bioremediation. Environment International 33, 406-413

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Eigenständigkeitserklärung

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René Phieler, Annekatrin Voit, Erika Kothe (2014): Microbially supported phytoremediation of heavy metal contaminated soils: Strategies and applications. Geobiotechnology I. Advances in Biochemical Engineering/Biotechnology. Volume 141. pp. 211-235. Springer-Verlag Berlin Heidelberg

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